ELSEVIER
Food Chemistry 69 (2000) 161–165
Food Chemistry
www.elsevier.com/locate/foodchem

# Thermal behavior and hydration properties of yeast proteins from *Saccharomyces cerevisiae* and *Kluyveromyces fragilis*

Miguel A. Otero[a,*], Jorge R. Wagner[b], María C. Vasallo[a], Lourdes García[a], María C. Añón[b]

[a]*Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar, (ICIDCA) Vía Blanca 804 Apdo. Postal 4026, La Habana 11000 Cuba*

[b]*Centro de Investigaciones y Desarrollo en Criotecnología de Alimentos (CIDCA) Facultad de Ciencias Exactas, Universidad Nacional de la Plata, Calle 47 y 116 (1900) La Plata, Provincia de Buenos Aires, Argentina*

Received 5 August 1998; accepted 15 October 1999

**Abstract**

High pressure homogenization of yeast cells followed by incubation at 50°C for the dissociation of ribonucleic acid-protein complexes resulted in a high denaturation degree of isolated proteins. Proteins in intact cells exhibited an ample endothermic peak with peak temperatures ($T_P$) at 66.66 and 63.67°C for *S. cerevisiae* and *K. fragilis*, respectively. No differences were found with respect to the associated enthalpy changes for both studied species. The isolation of proteins from its biomass shifts $T_P$ to values around 50°C. Impurities such as nucleic acid, polysaccharides and other intracellular components seem to play a protective role upon denaturation. Isolated proteins showed solubilities lower than 40% but exhibited water retention properties and wettability from 3.5 to 7.0 ml of water/g of protein. © 2000 Elsevier Science Ltd. All rights reserved.

## 1. Introduction

Yeast and yeast derivatives have been widely used in the formulation of food systems. Ethanol production and bakery account for the most widespread applications. Yet, yeast biomass is also rich in protein, polysaccharides and other commercially important compounds that can be isolated for the upgrading of yeast production (Dziezak, 1987).

The high content of nucleic acids and poor functionality have limited, up to date, the utilization of the whole biomass for protein enrichment in foods, due to the potential risks, concomitant to its intake, on human health (Edozien, Udo, Young & Scrimshaw, 1970). The isolation of yeast proteins is an attractive alternative for the upgrading of yeast biomass through its use as emulsifying, gelling, foam stabilizer agents etc. in food systems (Dziezak, 1987).

Yeast protein isolation has to be carried out through the breakdown of cell integrity. Several methods have been proposed for the disintegration of microbial cells but quite a few have proven to be efficient enough at large scale. Among them, homogenization has been reported as the most suitable method for this purpose (Baldwin & Robinson, 1992; Harrison, 1991; Harrison, Dennis & Chase, 1991; Middleberg, O'Neill & Bogle, 1991). However, homogenization is a high energy-consuming operation (Chisty & Moo-Young, 1986), thus the reduction of disruption steps is desirable to improve process economics.

It has already been demonstrated, that a mild alkaline medium allows the reduction of the homogenization stage from 5 steps to 2 without significant changes in the amount of extracted proteins (Otero, Vasallo, Verdecias, Fernández & Betancourt, 1996). In that sense, combined treatments seem to be convenient for this purpose. Additionally, the reduction of homogenization steps prevents debris micronization, a potential source of troubles during downstream processing.

There is scarce information about yeast protein functionality (Huang & Kinsella, 1987; Otero et al., 1996; Shetty & Kinsella, 1986) and more knowledge is needed to assess their potentialities as food ingredients.

Main functional properties are related to their interaction with water. Solubility provides an index of native structure and it is highly desirable for liquid beverages,

* Corresponding author.

0308-8146/00/$ - see front matter © 2000 Elsevier Science Ltd. All rights reserved.
PII: S0308-8146(99)00245-9

emulsions and foam formation and stabilization (Fligner & Mangino, 1991). In turn, water binding is important for doughs and batters formulation.

Yeast proteins are ubiquituous inside the cell and are released to the surroundings once the external structures are disrupted. An important fraction of these proteins is bound to ribonucleic acids as nucleoproteins (Huang & Kinsella, 1987) that have to be cleaved to obtain protein isolates. Several methods have been proposed to reduce nucleic acid content in yeast protein isolates but they exhibit several disadvantages with respect to nutritional properties and structure, which could lead to the impairment of their functionality (Bueno, Otero, Klibansky & González, 1985; Damodaran & Kinsella, 1984; Otero et al., 1996). The activation of intracellular RNase is a fast and efficient procedure to reduce RNA both in whole or disintegrated yeast cells (Bueno et al., 1985; Maul, Sinskey & Tannenbaum, 1970) but can also result in proteolytic degradation (Damodaran & Kinsella, 1983). The aim of the present paper is the study some functional and thermal properties of yeast protein isolates at a variety of treatment conditions and their comparison with non-isolated proteins in intact cells.

## 2. Materials and methods

### 2.1. Micro-organisms

*Saccharomyces cerevisiae* purchased in the local market as instant active dry yeast (Red Star, Levaduras Universal, Guatemala) and *Kluyveromyces fragilis* L-1930 were used in this study. The later was propagated in continuous mode on a fully-instrumented Marubishi MD100 fermentor with sugar cane molasses (40 mg/ml of total reducing substances) as carbon and energy sources. The culture broth was supplemented with diamonium phosphate (1.60 mg/ml) and diamonium sulfate (5.60 mg/ml) as phosphorus and nitrogen sources respectively. Growth was conducted at $D(=\mu)=0.25$ $h^{-1}$, $T=32°C$, pH 4.5 and oxygen transfer rate of 80 mM/lh. Cells were harvested by centrifugation (Sharpless Open Type 1A, Tumba, Sweden), washed twice with distilled water and stored at 4°C until use.

### 2.2. Protein isolation

The isolation scheme proposed in a previous paper (Otero et al., 1996) was modified to achieve different treatment alternatives specially at incubation and precipitation stages. Yeast suspensions (150 g/l) adjusted at pH 9,5 with 0.1 N NaOH, were homogenized twice at 50 MPa, diluted to 100 g/l and centrifuged to remove cell debris. Supernatants were submitted to different treatments. Samples were incubated at 50°C for 1 h in the presence of 0.05 M ethylene-diamino-tetraacetic acid (EDTA) (Lurton, Segain & Feuillat, 1989), and in its absence followed by isoelectric precipitation at pH = 4.5 in all cases. Additional samples were incubated without EDTA but precipitation was aided by coagulation at 90°C for 15 min. Finally, samples were directly precipitated at pH 4.5 without incubation. Table 1 shows the summary of treatment conditions.

### 2.3. Differential scanning calorimetry

Samples (10–15 mg) of 30% dispersions in water were hermetically sealed in aluminium pans and heated from 10 to 110°C at a heating rate of 10°C/min in a rheometric scientific calorimeter (Piscataway, NJ). A double empty pan was used as reference. Denaturation enthalpies (J/g), $T_{ONSET}$ and $T_P$ were drawn from obtained thermograms. Enthalpies ($\Delta H$) were expressed as J/g corrected for dry weight by perforating the pans and heating overnight at 105°C (Wagner & Añón, 1990). All assays were performed in duplicate.

### 2.4. Protein solubility and water holding capacity (WHC)

Protein isolates were dispersed (1%) in 0.01M phosphate buffer (pH 7.0) and stand still at 25°C for 1 h with occasional stirring. Samples were then centrifuged at 10,000 *g* for 30 min at 15°C. Protein content in supernatant was determined by Biuret (Gornall, Bardawill & David, 1949). The WHC of insoluble fraction was determined by subtracting protein in supernatant to those originally placed in tube and was expressed as millilitres of water per gram of protein. Assays were made by duplicate.

Table 1
Isolation schemes for yeast proteins from *Saccharomyces cerevisiae* and *Kluyveromyces fragilis* species[a]

| Treatment conditions | Precipitation | Kjeldahl protein, % | RNA content % |
|---|---|---|---|
| No incubation (I) | pH 4.5 | 76.45 ± 0.25a<br>81.79 ± 0.32c | 9.18 ± 0.09a<br>13.61 ± 0.11e |
| 50°C, 1 h<br>0.05 mM EDTA (II) | pH 4.5 | 76.50 ± 0.11a<br>79.09 ± 0.19d | 7.18 ± 0.16b<br>14.45 ± 0.18f |
| 50°C, 1 h (III) | pH 4.5 | 73.32 ± 0.27a<br>81.72 ± 0.46c | 3.48 ± 0.24c<br>16.98 ± 0.08g |
| 50°C, 1 h (IV) | pH 4.5, 90°C<br>15 min | 84.07 ± 0.13b<br>83.04 ± 0.21c | 1.20 ± 0.12d<br>16.20 ± 0.19h |

[a] All values are referred to dry matter (mean ± confidence interval). Values in the same column bearing different letters are significantly different ($P \leqslant 0.05$).

### 2.5. Water imbibing capacity (WIC)

The WIC of yeast proteins were determined by duplicate by means of a modification of the Baumann apparatus (Torgensen & Toledo, 1977) as previously described (Sorgentini, Wagner & Añón, 1995). WIC was expressed as millilitres of water per gram of protein.

### 2.6. Chemical analysis

Nitrogen was estimated according to Kjeldahl in a 1030 Kjeltec auto system (Tecator AB, Höganäs, Sweden). Nucleic acids were determined by hot perchloric extraction and reading at 270 and 290 nm (Rut, 1973).

## 3. Results and discussion

Table 1 shows the treatment conditions assayed for the isolation of yeast proteins as well as their Kjeldahl protein and nucleic acid content. Proteins from *K. fragilis* exhibited a higher nitrogen content than those from *S. cerevisiae* due to the increased amounts of protein-bound nucleic acids. A less efficient RNA degradation becomes evident for *K. fragilis*. In fact, incubation conditions established were optimal for *S. cervisiae* and no previous study was conducted with the former specie. The table shows significant differences among samples as a result of the treatment conditions. According to the biomass composition of both species, these results suggest protein content in isolated samples closely related with that in intact cells. Although both species may achieve a similar composition under the same culture conditions, baker's yeast is intentionally produced low in protein for a better conservation.

Thermograms of whole cells obtained by DSC are shown in Fig 1. They exhibited a similar endothermic transition showing the high similarities in thermal behavior of all proteins. However, the endotherm belonging to *S. cerevisiae* seems to have more cooperativity than that corresponding to *K. fragilis* as it is demonstrated by the small shoulder between 50 and 60°C, that cannot be separated in individual peaks by this technique. Those results are quite different to that reported for soy flour in which two peaks corresponding to glycinin and β-conglicinin are clearly differentiated (Arrese, Sorgentini, Wagner & Añón, 1991). Temperature peaks are $66.66 \pm 0.09$°C and $63.67 \pm 0.10$°C for *S. cerevisiae* and *K. fragilis* respectively, which suggests a slightly higher thermal resistance from the former. The $\Delta H$ associated with these changes revealed no differences between both species and resulted in lower ones than those reported for other plant proteins (Arrese et al., 1991; Wagner & Añón, 1990) and living spores.

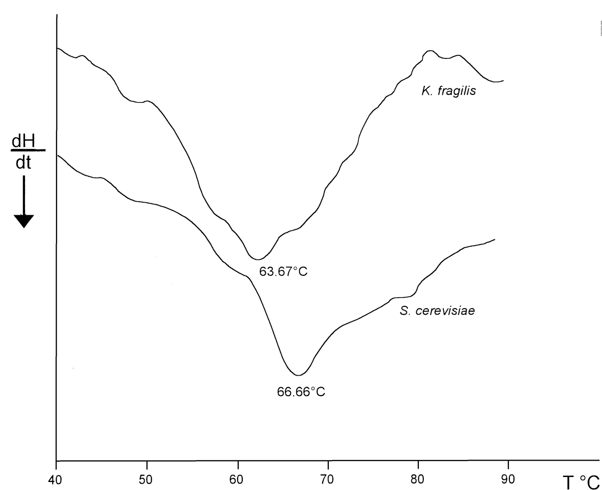


Fig. 1. Differential scanning calorimetry of intact cells from *Saccharomyces cerevisiae* (baker's yeast) and *Kluyveromyces fragilis*.

When proteins are isolated from its natural environment, a shift in peak temperature became evident. Protein samples show $T_P$ values around 50°C, well below from those obtained for intact cells (Table 1). This result appears to be related to the protective environment achieved through protein interactions with other biomolecules as polysaccharides and nucleic acids (Huang & Kinsella, 1987). This is also exhibited for $\Delta H$ values in isolated proteins where high denaturation degrees were observed as compared with intact cells (Fig. 1). Denaturation starts ($T_{onset}$) for isolated proteins at temperatures around 50°C, significantly lower than those observed for non-disintegrated cells. The homogenization stage as such, implies an intrinsic temperature increase related to adiabatic compression in the inner chamber of homogenizer (Chisty & Moo-Young, 1986). If the temperature is not carefully controlled, its changes will allow protein denaturation in an important extent. On the other hand, nucleic acid degradation during incubation by intracellular RNase is carried out at temperatures close to 50°C, within the same range of $T_P$ and $T_{ONSET}$. As this step is carried out for 1 h to permit an extensive RNA degradation, additional denaturation effects have to be expected. It has to be taken into account the probability of protease activation which could be another source for denaturation (Damodaran & Kinsella, 1983; Lurton et al., 1989). However, protease action could be reduced by the addition of protease inhibitors. As most yeast proteases are $Mg^{2+}$-dependent metalloproteins (Lurton et al., 1989) it could be expected, that the addition of EDTA a well-known complexing agent, may result in a decrease of protease activity, in case it exists.

Fig. 2 shows $\Delta H$ values for proteins isolated under different conditions. All samples exhibited a high denaturation degree (estimated from those obtained for intact cells). Yet, certain differences among them are evident. Less denaturing condition is the direct precipitation of

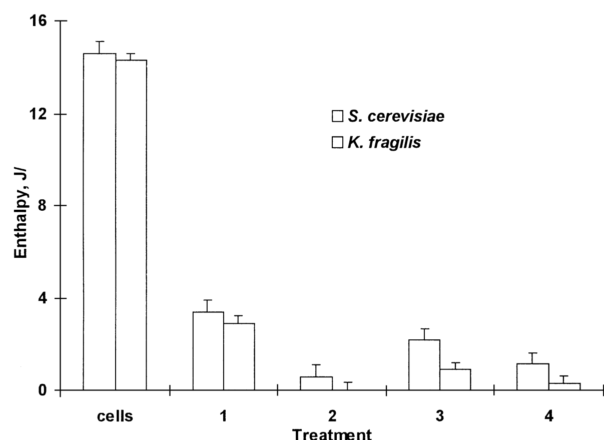


Fig. 2. Denaturation enthalpies ($\Delta H$, J/g) of yeast proteins isolated by high pressure homogenization. Enthalpy values were calculated per weight of protein.

full-RNA yeast proteins (non-incubated samples) which presented higher residual enthalpies than expected since nucleoprotein can be stabilized by non-covalent forces as electrostatic interactions and hydrogen binding which lead to an increased thermal resistance (Shetty & Kinsella, 1982). The effect of temperature seems to induce important changes in protein structure even in the absence of EDTA. Protein coagulation also leads to almost totally denatured proteins.

Table 2 shows denaturation temperatures of yeast proteins. In general, denaturation starts below 50°C, which suggests that incubation is the main denaturing factor. Yeast RNases exhibit optimal temperatures very close to this value (Bueno, Otero & González, 1982), yet, this step has to be carried out at lower temperatures and higher retention times to prevent thermal denaturation. Other methods such as the use of chaotropic salts (Damodaran & Kinsella, 1984), have to be studied as well, in connection with protein denaturation kinetics.

Table 2
Denaturation temperatures and final pH of yeast protein isolates from *Saccharomyces cerevisiae* and *Kluyveromyces fragilis* obtained by homogenization under different conditions[a]

| Treatment | $T_{onset}$, °C | $T_p$, °C | pH |
|---|---|---|---|
| *S. cerevisiae* cells | 56.15 ± 0.22a | 66.65 ± 0.09a | 6.00 |
| I | 51.38 ± 0.18a | 59.04 ± 0.18b | 6.33 |
| II | 47.21 ± 0.31b | 50.99 ± 0.21c | 2.88 |
| III | 46.54 ± 0.30c | 50.92 ± 0.21d | 5.59 |
| IV | 46.99 ± 0.16d | 51.28 ± 0.09e | 5.27 |
| *K. fragilis* cells | 55.60 ± 0.11e | 64.67 ± 0.10f | 5.60 |
| I | 45.34 ± 0.23f | 50.37 ± 0.25g | 6.70 |
| II | ND | ND | 2.80 |
| III | 45.04 ± 0.13g | 49.47 ± 0.19h | 5.22 |
| IV | 37.75 ± 0.13h | 48.41 ± 0.15 | 5.26 |

[a] All values are referred to dry matter (mean ± confidence interval). Values in the same column bearing different letters are significantly different ($P \leqslant 0.05$).

In Table 2, pH of 2% solutions of all samples is also offered. It can be observed that all samples remained in the acidic range, specially those samples treated with EDTA which achieved a very low pH after lyofilization. As samples were not neutralized before drying, the effect of pH lowering by the free carboxyl groups of EDTA probably increased the denaturing effect of the environment.

It is observed that *S. cerevisiae* proteins are slightly less sensitive to thermal denaturation than those from *K. fragilis*. It might also be argued that the lower protein content in intact cells leads to a less pure isolate with its concomitant stabilizing effect.

Table 3 shows some properties related to protein–water interactions. Solubility is affected by the loss of native structure and drops in all samples below 40%. Denaturation affected solubility by protein unfolding and subsequent aggregation (Pauly, Heinrichs & Luck, 1996). It has to be noticed that, despite the fact that nucleoproteins have been reported as intrinsically non-soluble compounds (Kinsella, 1986), in our results they were the most soluble samples probably due to their milder isolation conditions. As a result of solubility losses samples hydrated easily. Huang and Kinsella (1986), reported WHC values for yeast nucleoproteins in the range of 5–10 ml of water/g of protein.

Protein from plant sources such as soybean, cottonseed and sunflower exhibited WHC from 2.5 to 6.0 ml of water/g of protein (Huang & Kinsella, 1986) lower than those reported in this paper.

When both protein sources were compared with respect to swelling properties (WHC) a random pattern was observed. Whereas *S. cerevisiae* nucleoproteins have a higher water retention than their homologues from *K. fragilis* and similar results were obtained for samples incubated in presence of EDTA, the opposite is obtained for the other treatments. No explanation for such a behavior was found, either through drying pH or residual $\Delta H$.

Table 3
Solubility, water holding capacity (WHC) and water imbibing capacity (WIC) of yeast proteins from *S. cerevisiae* and *K. fragilis* cells[a]

| Sample | Solubility, % | WHC, ml/g | WIC, ml/g |
|---|---|---|---|
| *Saccharomyces cerevisiae* | | | |
| I | 36.60 ± 0.23a | 6.78 ± 0.12a | 3.29 ± 0.04a |
| II | 24.16 ± 0.25b | 4.40 ± 0.07b | 3.85 ± 0.09b |
| III | 17.85 ± 0.41c | 5.64 ± 0.09c | 1.71 ± 0.11c |
| IV | 24.51 ± 0.28d | 5.20 ± 0.19d | 3.17 ± 0.10d |
| *Kluyveromyces fragilis* | | | |
| I | 37.42 ± 0.11e | 6.23 ± 0.16e | 3.94 ± 0.14e |
| II | 27.90 ± 0.52f | 3.58 ± 0.16f | 2.59 ± 0.15f |
| III | 22.54 ± 0.43g | 6.91 ± 0.11g | 1.80 ± 0.06g |
| IV | 28.50 ± 0.38h | 6.94 ± 0.12h | 4.79 ± 0.08h |

[a] All values are referred to dry matter (mean ± confidence interval). Values in the same column bearing different letters are significantly different ($P \leqslant 0.05$).

## 4. Conclusions

DSC thermograms of intact yeast cells give endothermic peaks with $T_P$ around 65°C. Significant differences were found between the studied species with respect to $T_{ONSET}$ or $T_P$ but not for the associated enthalpy. Yeast proteins isolated by homogenization-incubation exhibited a high denaturation degree with respect to those in intact cells with a denaturation temperature around 50°C. Incubation resulted in being the main factor affecting protein denaturation, so it should be avoided. Protein precipitated by isoelectric pH have to be neutralized before drying to prevent further denaturation. Swelling properties in excess or limited water environments are similar to those found for other plant proteins.

## Acknowledgements

This paper was partially supported by a grant (RGA No. 96-146) from Third World Academy of Sciences.

## References

Arrese, E. L., Sorgentini, D. A., Wagner, J. R., & Añón, M. C. (1991). Electrophoretic, solubility and functional properties of commercial soy protein isolates. *Journal of Agricultural and Food Chemistry, 39*, 1029–1032.

Baldwin, C., & Robinson, C. W. (1992). Increased disruption resistance of *Candida utilis* grown for survivors of enzymatic lysis combined with high pressure homogenization. *Letters in Applied Microbiology, 15*, 59–62.

Bueno, G. E., Otero, M. A., & González, A. C. (1982). Characterization of intracellular RNase in heat-processed yeast cells. *Biotechnology Letters, 4*, 145–148.

Bueno, G. E., Otero, M. A., Klibansky, M. M., & González, A. C. (1985). Nucleic acid reduction from yeast: activation of intracellular RNase. *Acta Biotechnologica, 5*, 91–100.

Chisty, Y., & Moo-Young, M. (1986). Disruption of microbial cells for intracellular products. *Enzyme and Microbial Technology, 8*, 194–204.

Damodaran, S., & Kinsella, J. E. (1983). The use of chaotropic salts for separation of ribonucleic acid and proteins from yeast nucleoproteins. *Biotechnology and Bioengineering, 25*, 761–770.

Damodaran, S., & Kinsella, J. E. (1984). Dissociation of yeast nucleoprotein complexes by chemical phosphorylation. *Journal of Agricultural Food Chemistry, 32*, 1030–1037.

Dziezak, J. (1987). Yeast and yeast derivatives: applications. *Food Technology, 32*, 122–124.

Edozien, J. C., Udo, U. U., Young, V. R., & Scrimshaw, N. S. (1970). Effect of high levels of yeast feeding on uric acid metabolism of young men. *Nature (London), 228*, 180.

Fligner, K.L., & Mangino, M.E. (1991). Relationship of composition and protein functionality. In K.L. Fligner & M.E. Mangino, *Interactions of food proteins* (pp. 1–12). American Chemical Society.

Gornall, A. C., Bardawill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the Biuret reaction. *Journal of Biological Chemistry, 177*, 751–766.

Harrison, S. T. L. (1991). Bacterial cell disruption: a key unit operation in the recovery of intracellular products. *Biotech Adv., 9*, 217–240.

Harrison, S. T. L., Dennis, J. S., & Chase, H. A. (1991). Combined chemical and mechanical processes for the disruption of bacteria. *Bioseparation, 2*, 95–105.

Huang, Y. T., & Kinsella, J. E. (1986). Functional properties of phosphorylated yeast protein: solubility, water holding capacity and viscosity. *Journal of Agricultural and Food Chemistry, 34*, 670–674.

Huang, Y. T., & Kinsella, J. E. (1987). Effect of phosphorylation on emulsifying and foaming properties and digestibility of yeast proteins. *Journal of Food Science, 52*, 1684–1688.

Kinsella, J. E. (1986). Functional properties from yeast nucleoproteins for food uses. In D. Knorr, *Methods for isolation in food biotechnology* (pp. 363–380). New York: Marcel Dekker.

Lurton, L., Segain, J. P., & Feuillat, M. (1989). Proteolysis during the autolysis of yeast under acidic conditions. *Sciences des Aliments, 9*, 111–123.

Maul, S. B., Sinskey, A. J., & Tannenbaum, S. R. (1970). New process for reducing the nucleic acid content of yeast. *Nature (London), 228*, 181.

Middleberg, A. P. J., O'Neill, B. K., & Bogle, I. D. L. (1991). Combined chemical and mechanical processes for the disruption in high-pressure homogenization: studies on *E. coli* containing recombinant bodies. *Biotechnol Bioeng., 38*, 363–370.

Otero, M. A., Vasallo, M. C., Verdecia, O., Fernández, V. M., & Betancourt, D. (1996). Whole fractionation of baker's yeast. *Journal of Chemical Technology and Biotechnology, 67*, 67–71.

Pauly, D., Heinrichs, M., & Luck, T. (1996). Evaluation of the denaturation kinetics of two differently prepared protein concentrates from bovine blood plasma. *Journal of Agricultural and Food Chemistry, 44*, 3251–3256.

Rut, M. (1973). Determination of nucleic acid on yeast and yeast-related products. *Kvasny Prumysl, 19*, 131–133.

Shetty, J. K., & Kinsella, J. E. (1982). Isolation of yeast proteins with reduced nucleic acid level by using reversible acylating agents. Some properties of the isolated proteins. *Journal of Agricultural and Food Chemistry, 30*, 1162–1171.

Sorgentini, D. A., Wagner, J. R., & Añón, M. C. (1995). Effect of thermal treatment of soy protein isolate on the characteristics and structure. Function relationship of soluble and insoluble fractions. *Journal of Agricultural and Food Chemistry, 43*, 2471–2479.

Torgensen, H., & Toledo, R. T. (1977). Physical properties of protein preparation related to their functional characteristics in comminuted heat systems. *Journal of Food Science, 42*, 1615–1620.

Wagner, J. R., & Añón, M. C. (1990). Influence of denaturation degree, hydrophobicity and sulfhydryl content on solubility and water absorbing capacity of soy protein isolates. *Journal of Food Science, 50*, 750–770.