DEVELOPMENT AND CHARACTERIZATION OF EDIBLE FILMS BASED ON GLUTEN FROM SEMI-HARD AND SOFT BRAZILIAN WHEAT FLOURS (DEVELOPMENT OF FILMS BASED ON GLUTEN FROM WHEAT FLOURS)[1]

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SUMMARY

Edible films based on gluten from four types of Brazilian wheat gluten (2 "semi-hard" and 2 "soft") were prepared and mechanical and barrier properties were compared with those of wheat gluten films with vital gluten. Water vapor, oxygen permeability, tensile strength and percent elongation at break, solubility in water and surface morphology were measured. The films from "semi-hard" wheat flours showed similar water vapor permeability and solubility in water to films from vital gluten and better tensile strength than the films from "soft" and vital gluten. The films from vital gluten had higher elongation at break and oxygen permeability and also lower solubility in water than the films from the Brazilian wheat "soft" flours. In spite of the vital gluten showed greater mechanical resistance, desirable for the bakery products, for the purpose of developing gluten films Brazilian "semi-hard" wheat flours can be used instead of vital gluten, since they showed similar barrier and mechanical properties.

Keywords: mechanical properties; permeability; vital gluten.

RESUMO

DESENVOLVIMENTO E CARACTERIZAÇÃO DE FILMES COMESTÍVEIS DE GLÚTEN DE FARINHAS FORTES E FRACAS DE TRIGOS BRASILEIROS. Filmes à base de glúten de quatro tipos de farinhas de trigo brasileiras (2 "semi-fortes " e 2 "fracas") foram preparados e suas propriedades mecânicas e de barreira foram comparadas com filmes com glúten vital (comercial). Permeabilidade ao vapor d'água e oxigênio, resistência à tração, porcentagem de elongação na ruptura, solubilidade em água e morfologia de superfície foram medidas. Filmes de glúten das farinhas "semi-fortes" mostraram similar permeabilidade ao vapor d'água e solubilidade em água em comparação aos filmes de glúten vital e melhor resistência à tração do que os filmes das farinhas "fracas" e glúten vital. O filme de glúten vital apresentou maior elongação na ruptura e permeabilidade ao oxigênio do que os filmes das farinhas brasileiras e ainda mais baixa solubilidade que as farinhas fracas. Apesar do glúten vital ter uma grande resistência mecânica, desejável para produtos de panificação, para o propósito do desenvolvimento de filmes comestíveis as farinhas de trigo "semi-duras" brasileiras podem ser usadas ao invés do glúten vital, já que elas mostraram propriedades de barreira e mecânicas similares.

Palavras-chave: propriedades mecânicas; permeabilidade; glúten vital.

1 – INTRODUCTION

Recently interest in edible films and coatings for foods has grown considerably [1, 21, 24, 35]. Such films can control mass transport between the components of a product, as well as between the product and its surrounding environment [12]. The kinetics of chemical and enzymatic reactions, microbial growth, texture behavior and the physical stability of foods are all strongly influenced by their moisture contents and this can vary drastically as a result of processing, packaging and shelf life conditions [19]. Other factors contributing to renewed interest in the development of edible films include consumer demand for high quality foods, environmental concerns over the disposal of non-renewable food packaging materials, and opportunities for creating new market outlets for film-forming ingredients derived from agricultural products.

Polysaccharides, proteins and lipids can be used as edible film-forming agents [5, 13, 24, 30]. The use of proteins for the preparation of edible films has been increased in the past decade [14, 37]. They show advantageous properties in the preparation of biofilms due to their ability to form networks which improves the barrier properties of the films. Additionally, due to the characteristics of the protein orientation in the matrix of the films, mechanical properties like plasticity and elasticity can be obtained. Different kinds of vegetal and animal origin proteins have been used, such as soy proteins [7], wheat gluten [20], cotton seed protein [28], peanuts, corn zein and peas [10, 22], whey proteins [42] and gelatin [41].

Due to the unique cohesive and elastic properties of gluten [44], good film-forming properties may be expected. Biodegradable and edible films from wheat proteins are useful in food packaging provided they are flexible, strong, heat sealable, and relatively transparent [36]. The mechanical and barrier properties of wheat gluten films have been studied [9, 14, 18]. An edible wheat gluten film was developed and the effects of gluten and ethanol concentrations and pH of the film-forming solution on various film properties, were evaluated [17]. HERALD *et al.* [23] compared wheat gluten films prepared at various pH values observed that films prepared at pH 3.3 showed higher water vapor permeability, presumibly due to the unfolding conformation of protein molecules and exposure of hydrophilic groups. Edible wheat gluten films were prepared with various amounts of glycerol [43]. Films with low amounts of glycerol had lower water vapor and oxygen permeability, higher tensile strength and lower elongation at break. Our objectives were to develop edible gluten films from four types of Brazilian wheat (2 "semi-hard" and 2 "soft") and compare them with some

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functional properties of wheat gluten films prepared with vital gluten, which is the product usually used in research involving edible gluten. The absence of studies about edible films from some varieties of wheat flours can justify this work.

2 – MATERIALS AND METHODS

2.1 – Materials

Vital wheat gluten (Rhodia, Campinas, Brazil), glycerol (Merck, Darmstadt, Germany), ammonium hydroxide (Synth, São Paulo, Brazil), calcium chloride (ECIBRA, São Paulo, Brazil), sodium chloride (Synth, São Paulo, Brazil), magnesium nitrate (ECIBRA, São Paulo, Brazil), solid paraffin (Chemco, São Paulo, Brazil), 2 types of Brazilian "semi-hard" wheat (EMBRAPA 22 and EMBRAPA 42) and 2 types of Brazilian "soft" wheat flour (Trigo BR 23 and EMBRAPA 119) from EMBRAPA (Rio Grande do Sul, Brazil) were used in this study. "Semi-hard" wheat EMBRAPA 22 is "harder" than EMBRAPA 42 and the "soft" wheat EMBRAPA 119 is "harder" than Trigo BR 23.

2.2 – Gluten extraction

The wheat was milled in a Brabender mill (Quadrumat Senior, OHG Duisburg, Germany) to obtain the wheat flour. The gluten was extracted from the wheat flour (200g) by adding distilled water and mixing vigorously to form a mass. This mass was washed exhaustively until the water became transparent, the remaining mass being the gluten. The gluten was dried at 40^{0}C in a vacuum oven (20 in Hg) for 24h, broken up in a blender (Philips, São Paulo, Brazil) and ground in a small Brabender mill (type 881400, OHG Duisburg, Germany) to obtain gluten powder.

2.3 – Gluten tensile strength and extensibility

The gluten from the "hard" and "soft" wheat flours was prepared in the Glutomatic (Gluten Index type 2200, Perten Instruments, Huddinge, Sweden), according to SMEWING [40]. These glutens and the vital gluten were transferred to 50mL centrifuge tubes, distilled water added (in a quantity sufficient to cover the samples) and the tubes then centrifuged at 2789g for 5 minutes at room temperature to disrupt any bubbles formed during the process. The gluten was then transferred to a convenient mold and allowed to rest for 40 minutes at 30^{0}C. The tensile strength (TSg) and extensibility of the gluten were then measured in the Texture Analyzer TA.XT2 (Stable Micro System, Surrey, UK). The probe used was specifically for the gluten extensibility test (dough and gluten extensibility rig) and the cross head speed was 3.3mm/s. Six samples of each kind of gluten were measured.

2.4 – Film preparation

The film was prepared from a solution of gluten (7.5g/100mL solution), absolute ethanol (45.0mL/100mL solution), glycerol (1.12 or 1.50g/100mL solution), ammonium hydroxide (to adjust to pH 10.0) and distilled water. All components were mixed and heated using a magnetic stirrer until the temperature of the mixture reached 70^{0}C. The solution was then centrifuged (50mL centrifuge tubes) at 5856g for 6 min at room temperature. The film-forming solution was poured and spread evenly over a Teflon covered glass surface and dried at room temperature for 24h (modified method of GONTARD *et al.* [18]). The quantity of solution poured onto the surface was calculated to obtain a constant thickness of the dried film. All films used for experiments were equilibrated at 52% RH (saturated $Mg(NO_3)_2$ solution in the dessicator) at 25^{0}C for 48h before being tested.

2.5 – Film thickness

Film thickness was measured using a micrometer (Model MDC-25M, Mitutoyo, MFG, Japan). The thickness of individual film samples was determined as a random average of five measurements.

2.6 – Water vapor permeability

The water vapor transmission rate of the films was determined gravimetrically at 25^{0}C using a modified American Society for Testing and Materials Standard Method E-96 [4] and the water vapor permeability (WVP) was calculated according to this method. The samples, triplicates of each film, were conditioned for 2 days at 52% RH before measurement. The relative humidity gradient of the test was 0% RH ($CaCl_2$ desiccant in the test cup) and 75% RH (saturated NaCl solution in the desiccator) on both sides of the film.

2.7 – Oxygen permeability

The oxygen transmission rates were determined using a modification of the ASTM Standard Method D 3985-81 [2] with an Ox-Tran apparatus (Mocon, Inc., Minneapolis, USA) at dry condition and 25^{0}C. The samples, duplicates of each film, were conditioned for 2 days at 52% RH before measurement. The oxygen permeability (OP) was calculated by dividing the oxygen transmission rate by the oxygen pressure and multiplying by the mean thickness.

2.8 – Tensile strength and percent elongation at break

Film tensile strength (TS) and percent elongation at break (ELO) were determined using a Texture Analyzer TA.XT2 (Stable Micro System, Surrey, UK), operated according to the ASTM [3] Standard Method D 882-83 (initial grip separation = 50mm and cross head speed = 100mm/min). Six specimens (100mm long and 25.4mm wide) of each film were measured. The peak loads and extension at break were recorded for tested film specimens. The tensile strength and percent elongation at break were calculated according to the ASTM method.

2.9 – Solubility in water

The percentage of initial dry matter of each film was determined at 105^{0}C for 24h. Two discs of film (2cm diameter) were cut, weighed, immersed in 50mL of distilled water and slowly and periodically agitated for 24h at 25ºC. The pieces of film were then taken out and dried (105ºC for 24h) to determine the weight of dry matter not solubilized by the water [18].

2.10 – Scanning electron microscopy

Film samples were examined for surface characteristics using a scanning electron microscope (Jeol, JMS-T330, Tokyo, Japan) operating at 10kV. Film samples were affixed to aluminum stubs with cupper tape and left in a desiccator with silica gel for 7 days. The samples were then sputter-coated with gold in a Balzers evaporator (SDC 050, Baltec, Lichtenstein, Austria) for 180 seconds at 40mA.

2.11 – Statistical analyses

The Statistica® (Microsoft, USA) program was used to calculate the analysis of variance (ANOVA) and a Tukey test was used to determine the significant differences of all film properties at a 95% confidence interval.

3 – RESULTS AND DISCUSSION

3.1 – Gluten

3.1.1 – Gluten tensile strength and extensibility

This test was carried out to investigate the resistance of the gluten, thus comparing the gluten tensile strength (TSg) of the vital (commercial) gluten and that of the glutens extracted from the wheat flours. The TSg of the gluten from the "soft" wheat flours (Trigo BR 23 and EMBRAPA 119) was indeed significantly lower than that of the gluten from the "semi-hard" wheat flours (EMBRAPA 22 and EMBRAPA 42) as can be seen in *Table 1*. However, there was no significant difference in TSg between the two types of "semi-hard" wheat flours, or between the "soft" flours. The TSg of the vital gluten was very high (279.50g), showing significant differences from the other glutens tested, which could indicate that this gluten was obtained from a "hard" wheat flour. On the other hand, its extensibility (4.82cm) was lower than the gluten from the "semi-hard" wheat flours (EMBRAPA 22 and EMBRAPA 42) and gluten from Trigo BR 23 (7.64cm). The gluten from EMBRAPA 22 and EMBRAPA 42 wheat flours had high extensibility (12.23 and 9.29cm, respectively) and EMBRAPA 119 had the lowest extensibility (1.90cm). These mechanical characteristics are important when the gluten is used in bakery products. The gluten strength for this purpose is determined by the gluten characteristics [31] including quantity of glutenin [39]; quantity of intermolecular disulfide bonds [15]; degree and extension of the arrange glutenin-gliadin [8] and quantity of the amino acid cysteine [11]. In the case of the films, the disulfide bonds are cleaved and reduced to sulphydryl groups when dispersing gluten in alkaline environment [32] associated with heating [25] and reformed during film drying in contact with air [15]. Studies with purified proteins of gluten indicate that glutenin produces stronger films than gliadin or vital gluten, this fact has been attributed to the larger and more extended nature of glutenin [26]. The extensive intermolecular interactions during the formation of the film result in brittle films, which require the addition of a plasticizer to increase film flexibility [18]. The combination of the gluten strength and quantity/type of plasticizer is important to obtain films with more adequate mechanical characteristics.

TABLE 1. Properties of resistance and extensibility of gluten

Type of gluten	Tensile strength (g)*	Extensibility (cm)*
Vital gluten	279.50 ± 26.78^a	4.82 ± 0.68^b
EMBRAPA 22	69.46 ± 3.08^b	12.23 ± 1.45^a
EMBRAPA 42	58.13 ± 3.03^b	9.29 ± 1.34^a
Trigo BR 23	21.38 ± 6.06^c	7.64 ± 2.92^a
EMBRAPA 119	22.17 ± 4.30^c	1.90 ± 0.29^c

*Mean and standard deviation of six replicates. a-d Means with different superscript letters in the same column are significantly different (P<0.05) according to the ANOVA and Tukey tests.

3.2 – Films

3.2.1 – Water vapor permeability

The water vapor permeability should be as low as possible since an edible film or coating should retard moisture transfer between the food and the environment, or between two components of a heterogeneous food product [17]. The films prepared with gluten extracted from both "soft" wheat flours (Trigo BR 23 and EMBRAPA 119) with a higher concentration of glycerol, showed higher values for WVP (15.37 and 14.65gmm/m²dKPa) as compared to films from vital gluten (11.35gmm/m²dKPa) and from the "semi-hard" wheat flours EMBRAPA 22 and EMBRAPA 42 (9.02 and 11.89gmm/m²dKPa, respectively), as shown in *Table 2*. As the glycerol concentration decreased from 25 to 15%, a decrease in the WVP values was observed, in agreement with other researchers [18, 43]. According to BANKER [6], plasticizers are added to films to reduce brittleness, increase toughness, strength, tear and impact resistance and impart flexibility. Usually, the addition of a plasticizer increase the permeability of gas, water vapor and solute and decreases the tensile strength of the films.

For the films with 15% glycerol, the WVP values were in the following order (*Table 2*): vital gluten < EMBRAPA 22 ("semi-hard") < EMBRAPA 42 ("semi-hard") < Trigo BR 23 ("soft") < EMBRAPA 119 ("soft"). Although the film from vital gluten had the lowest WVP, there were no significant differences (P<0.05) between the other films with 15% glycerol. The gluten films from Brazilian "semi-hard" wheat flours showed better barrier to water vapor properties than films from "soft" flours, and similar efficiency to those from vital (commercial) gluten.

3.2.2 – Solubility in water

Water resistance is an important property of edible films for applications in food protection, where water activity is high, or when the film must be in contact with water during processing of the coated food, to avoid exudation of fresh or frozen products. Therefore, edible films with high water solubility may be required, for example, to contain pre-measured portions, which will be dissolved in water or in hot food [21]. For all the films tested, the highest solubility in water was observed when the higher concentration (25% weight/weight of gluten) of glycerol was used. Films with glutens from the "soft" flours and 25% glycerol showed higher values for solubility in water and the film with vital gluten had the lowest value. For all the films with 15% glycerol, the values for solubility in water were not significantly different ($P<0.05$) and the lowest value was observed for the film with gluten from EMBRAPA 42 wheat flour ("semi-hard"). Increasing the amount of plasticizer, specially glycerol, caused an increase in moisture and WVP of the WPI (whey protein isolate) films [38]. This is probably due to the plasticizer that disrupts intermolecular interactions between polymer molecules [27]. Polyols seem to have ability to locate between polymer molecules, disrupting intermolecular polymer associations [18], being this effect attributed to their ability to associate with water. Significant differences in solubility in water for the films with higher concentration of glycerol were observed in the following order (*Table 2*): "soft" wheat > "semi-hard" wheat > vital gluten. Considering that glycerol is infinitely soluble in water [38], differences in solubility can be occurred due to the difference on the intensity of the polymer association, where films from "soft" wheat were not able to prevent the glycerol loss from the matrix to the aqueous solution. With lower concentration of glycerol, the solubility of the films was lower and there was no significant difference between all the films tested, indicating that the polymer matrix was able to partially accommodate and protect the polyol loss through the solution.

3.2.3 – Oxygen permeability

Good oxygen barrier properties of edible films are desired in food packaging and preservation. As an example, coating foods susceptible to lipid oxidation with protein films, in combination with an external, conventional moisture barrier package seems to be possible. Oxygen permeability of the films from wheat flour with the lower concentration of glycerol could not be measured because they were too brittle and cracked in the equipment. The film with gluten from EMBRAPA 22 "semi-hard" wheat flour had the lowest oxygen permeability ($24.26 cm^3 mm/m^2 dkPa$) of all the films tested. Oxygen permeability values (*Table 2*) of all the wheat gluten films tested (24.26-$39.67 cm^3 mm/m^2 dkPa$) were similar to the values obtained by Park and Chinnan (1990) for zein:glycerin films (13.0-$44.9 cm^3 mm/m^2 dkPa$) and gluten:glycerin films (9.6-$24.2 cm^3 mm/m^2 dkPa$) at 30^0C and 0% RH and were low compared to polysaccharide-based edible films (149.0-$910.0 cm^3 mm/m^2 dkPa$) and non-edible films like high density polyethylene ($427 cm^3 mm/m^2 dkPa$), maybe due to their polar nature and linear structure, which leads to a high cohesive energy density and a low free volume [43].

TABLE 2. The permeability of water vapor and oxygen and water solubility of films from vital gluten and gluten extracted from wheat flour ("semi-hard" and "soft")

Film	Water vapor permeability ($gmm/m^2 dKPa$)*	Solubility in water (%)*	Oxygen permeability ($cm^3 \mu m/m^2 dKPa$)*
Trigo BR23 25% glycerol	15.37 ± 1.10^{a}	71.1 ± 7.1^{a}	34.30 ± 1.25^{ab}
EMBRAPA 119 25% glycerol	14.65 ± 0.97^{ab}	55.8 ± 6.1^{ab}	35.14 ± 2.43^{a}
EMBRAPA 22 25% glycerol	9.02 ± 2.54^{bcd}	36.8 ± 1.8^{bcd}	24.26 ± 3.22^{b}
EMBRAPA 42 25% glycerol	11.89 ± 1.24^{abc}	39.3 ± 5.4^{bc}	36.88 ± 5.70^{a}
Vital gluten 25% glycerol	11.35 ± 0.35^{abc}	19.7 ± 0.6^{cd}	39.67 ± 1.41^{a}
Trigo BR23 15% glycerol	8.70 ± 1.02^{cd}	31.4 ± 0.8^{cd}	-
EMBRAPA 119 15% glycerol	9.56 ± 0.14^{bcd}	22.4 ± 1.0^{cd}	-
EMBRAPA 22 15% glycerol	7.29 ± 0.82^{cd}	23.4 ± 0.9^{cd}	-
EMBRAPA 42 15% glycerol	7.82 ± 0.16^{cd}	16.8 ± 0.3^{d}	-
Vital gluten 15% glycerol	5.72 ± 0.03^{d}	17.4 ± 0.2^{d}	35.82 ± 2.87^{a}

*Mean and standard deviation of replicates. $^{a\text{-}d}$ Means with different superscript letters in the same column are significantly different ($P<0.05$) according to the ANOVA and Tukey tests.

3.2.4 – Mechanical properties

An edible film should be resistant in order to withstand manipulation during its application and to maintain its integrity and also its barrier properties. *Table 3* shows that films prepared with glutens from "semi-hard" wheat flours (EMBRAPA 22 and EMBRAPA 42) and low concentrations of glycerol had greater tensile strength (7.80 and 10.89MPa, respectively) than films from other wheat flours (2.00 and 3.71MPa for Trigo BR 23 and EMBRAPA 119, respectively), vital gluten (2.36MPa) and gluten:glycerol (2.5:1) films from GENNADIOS *et al* ([14]; 2.60MPa), showing that films from this wheat flour had better tensile strength than the other films. The films from one of the "semi-hard" wheat flours (EMBRAPA 42) showed a higher percent elongation at break (184.20%) when compared to the films from the other Brazilian wheat flours (2.21-25.50%). All films with low amounts of glycerol had higher tensile strength and lower percent elongation at break, in agreement with TANADA-PALMU *et al* [43]. This similar behavior has been reported for other films [16, 29, 34]. Films with vital gluten and both concentrations of glycerol (15 and 25%) showed significantly higher percent elongation at break than the films made with gluten from Brazilian wheat flours.

TABLE 3. Mechanical properties of films from vital gluten and gluten extracted from wheat flour ("semi-hard" and "soft").

Film	Tensile strength (MPa)*	Elongation at break (%)*
Trigo BR23 25% glycerol	0.78 ± 0.23^{d}	24.20 ± 0.25^{b}
EMBRAPA 119 25% glycerol	0.91 ± 0.06^{cd}	25.50 ± 0.92^{b}
EMBRAPA 22 25% glycerol	1.39 ± 0.96^{cd}	13.96 ± 1.12^{b}
EMBRAPA 42 25% glycerol	2.41 ± 0.23^{cd}	184.20 ± 1.15^{a}
Vital gluten 25% glycerol	1.22 ± 0.47^{cd}	220.30 ± 1.56^{a}
Trigo BR23 15% glycerol	2.00 ± 0.28^{cd}	3.50 ± 2.00^{b}
EMBRAPA 119 15% glycerol	3.71 ± 0.67^{c}	14.00 ± 3.00^{b}
EMBRAPA 22 15% glycerol	7.80 ± 1.07^{b}	2.21 ± 1.56^{b}
EMBRAPA 42 15% glycerol	10.89 ± 1.56^{a}	4.81 ± 0.99^{b}
Vital gluten 15% glycerol	2.36 ± 0.02^{cd}	184.10 ± 8.40^{a}

*Mean and standard deviation of replicates. $^{a\text{-}d}$ Means with different superscript letters in the same column are significantly different ($P<0.05$) according to the ANOVA and Tukey tests.

3.2.5 – Scanning electron microscopy

The micrographs obtained from the film surfaces are presented in *Figures 1, 2* and *3.* The use of scanning electron microscopy to observe the morphology of the surface of the film was efficient in evaluating the films containing mixtures of polysaccharide-fatty acids or polysaccharide-beeswax and their functional properties [45]. In this case for films from different glutens, it was not possible to observe any significant morphological difference between "semi-hard" EMBRAPA 22 (*Figure 1*) and vital gluten (*Figure 3*), but the "soft" gluten film showed one more open matrix surface morphology which could be capable of explaining functional differences such as the worst barrier properties or mechanical resistance compared to the other films.

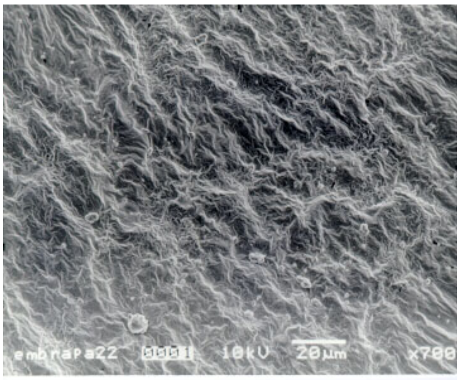

FIGURE 1. Micrograph of the film from a "semi-hard" gluten (EMBRAPA 22)

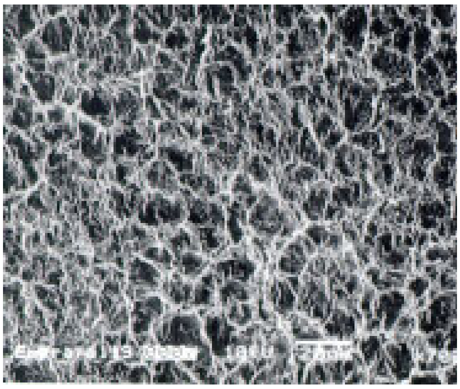

FIGURE 2. Micrograph of the film from a "soft" gluten (EMBRAPA 119)

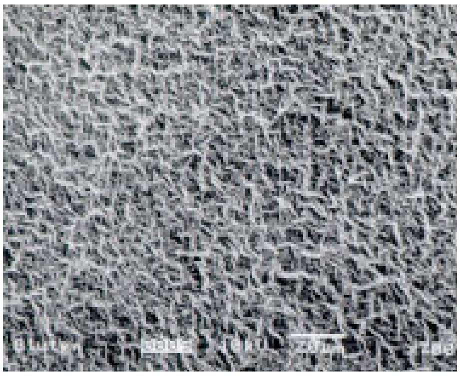

FIGURE 3. Micrograph of the film from the vital gluten

4 – CONCLUSIONS

Wheat gluten is a good source of protein-based biodegradable films. There was no significant difference between the gluten films obtained from the two types of Brazilian "semi-hard" wheat flours or between those obtained from the two "soft" flours. Films prepared with glutens from "semi-hard" wheat flour and the lower concentration of glycerol showed better properties as compared to films from the "soft" wheat flour. Brazilian "semi-hard" wheat flours can be used to develop edible gluten films instead of vital (commercial) gluten, since they showed similar barrier and mechanical properties. The possibility of improving the wheat gluten film properties by promoting crosslinking via enzymatic and chemical protein treatments, should be explored.

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6 – ACKNOWLEGMENTS

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