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Influence of Additives White Oat Flour and Barley Flour on Some Quality Characteristics of Yoghurt

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Abstract: This study aimed to evaluate the effect of adding different levels of white oat and barley flours (0.0, 0.5, 1.0, 1.5 & 2.0%) to Cow's milk for improving some functional properties of yoghurt during storage using SDS-PAGE electrophoresis analysis. Yoghurt was made from Cow's milk with the addition of both barley flour and white oat flour at levels of (0.0, 0.5, 1.0, 1.5 & 2.0%) of each alone, resultant of yoghurt of all treatments at 0 time , 7 days and 14 days was stored at 5°C. From the obtained results addition of barley or white oat flours at level up to 1.0% to yoghurt could be recommended.

Key words: SDS-PAGE - White Oat Flour - Barley Flour - Yoghurts

INTRODUCTION

Yogurt is a functional dairy product known for its therapeutic, nutritional and probiotic effects. It is produced by fermentation of milk with the thermophilic homofermentative lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *Bulgaricus* [1]. During recent years, an increasing interest has developed in foods that contribute to a positive effect on health beyond their nutritional value. Among these functional foods, much attention has been focused on probiotic products and food containing dietary fiber [2, 3]. Probiotics can be defined as living microorganisms that have proved beneficial effects on health of the host and that improve the intestinal microbial balance [4, 5]. Beneficial effects of probiotics include improving the gut microbial balance, stimulation of the immune system, reduction of blood cholesterol level and reduction in the incidence of cancer, cardiovascular diseases, diarrhea and osteoporosis [6, 7].

One of the approaches to increase the number of probiotic bacteria in the intestinal microbiota is including prebiotics in food systems, which are non-digestible dietary fiber components, mainly carbohydrates [8, 9]. Dietary fiber is naturally present in cereals, vegetables, fruits and nuts. Based on their simulated intestinal solubility, dietary fibers are either classified as soluble or insoluble fiber. Soluble fibers include pectin's, beta-glucans, galactomanan gums and a large range of no digestible oligosaccharides including inulin; insoluble fibers include lignin, cellulose and hemicelluloses [10, 11]. Foods rich in fiber components have high volume with low energy density and should promote satiation and satiety and play a role in the control of energy balance. These foods have the capacity of binding bile acids and metabolites of cholesterol that play an important role in digestion and absorption of lipids in the small intestine, lowering blood cholesterol, regulating blood glucose levels for diabetes management, producing short chain fatty acids and promoting the growth of beneficial gut microflora (i.e. as a prebiotic) [12, 13]. Due to beneficiary health effects the recommended daily intake of fiber is about 38 g for men and 25 g for women [14]. Fiber can be used for improvement of some functional properties such as texture, water holding capacity, oil holding capacity, emulsification and/or gel formation, bulking agent in reduced-sugar applications and shelf-life of processed foods [15, 16].

Polyacrylamide Gel Electrophoresis: (PAGE), describes a technique widely used in biochemistry, forensics, genetics, molecular biology and biotechnology to separate biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility. Mobility is a function of the length, conformation and charge of the molecule.

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As with all forms of gel electrophoresis, molecules may be run in their native state, preserving the molecules' higher-order structure, or a chemical denaturant may be added to remove this structure and turn the molecule into an unstructured linear chain whose mobility depends only on its length and mass-to-charge ratio. For proteins, sodium dodecyl sulfate (SDS) is an anionic detergent applied to protein samples to linearize proteins and to impart a negative charge to linearized proteins. This procedure is called SDS-PAGE. In most proteins, the binding of SDS to the polypeptide chain imparts an even distribution of charge per unit mass, thereby resulting in a fractionation by approximate size during electrophoresis. Proteins that have a greater hydrophobic content, for instance many membrane proteins and those that interact with surfactants in their native environment are intrinsically harder to treat accurately using this method, due to the greater variability in the ratio of bound SDS.

The study aimed to evaluate the effect of addition of different level of oat flour and barley flour to cow's milk for improving functional properties of yoghurt.

MATERIALS AND METHODS

Materials

Milk: Fresh Cow's milk with 12.26% TS, 3.51% fat, 3.42% protein, & 0.74% ash with 0.17% titratable acidity and pH 6.50 was obtained from a private farm in North Sinai Governorate.

Starter: Yoghurt culture (*Streptococcus thermophillus*, *Lactobacillus delbrueckii* ssp. *bulgaricus)* were obtained from DANISCO, Rue de Clemencieres-BP 32, Sassenage, Al- Denemark.

White Oats and Barley Flours: White Oat and Barley flours was obtained from Al Ghurair Foods Company of Dubai, United Arab Emirates.

Chemical Composition Analysis: Analyses were carried out as per standard methods. The crude protein content was calculated by multiplying the corresponding total nitrogen content, which was determined according to the Kjeldahl method (ICC standard method No.105/2)[17], by a factor of 6.25. Crude fat were extracted from the samples in a Soxhlet extractor with ether. The crude fat content was determined gravimetrically after oven-drying. Carbohydrates content has been determined according to Ewers polarimetric method (ISO 10520) [18], using the Elmer-Perkin polarimeter. Crude fiber content has been determined according to Kirschner-Ganakova‘s procedure [19]. To determine the ash content, the samples were placed in a muffle furnace at 900 °C for 2 hours and weighed before and after (ICC standard method No.104/1) [20].

SDS-PAGE: Electrophoresis was determined according to the method of Laemmli [21].

Methods

Manufacture of Yoghurt: Barley and white Oats flour were added were added to Cow's milk addition (0.0, 0.5, 1.0, 1.5 & 2.0%) and heated at 90°C/15 min, then cooled to 42°C; then inoculated with 3% yoghurt starter (*Streptococcus thermophillus, Lactobacillus delbrueckii ssp. bulgaricus*), then each sample was distributed into 100 mL in plastic cups, the cups were incubated at 42°C for 3 – 4 hours until a firm curd was formed. The resultant yoghurt was kept in a refrigerator (5°C) for 14 days.

Electrophoresis: Electrophoresis tests were done in Cairo University Research Park (CURP), Biotechnology department, Faculty of Agriculture, Cairo University.

Gradient gels are somewhat more difficult to pour and it may be worthwhile to spend the money on precast gels if the need for a gradient gel arises. This may be the case if you want to resolve a very broad range of molecular weights or for some reason the proteins you are interested in do not separate well using a straight percentage of acrylamide.

Now mix the ingredients needed for the chosen percentage and pour the solution quickly into your gel casting form - be sure to leave some room for the stacking gel - I usually leave about 2 centimeters below the bottom of the comb for the stacking gel. You can do this by inserting the comb into the dry form and marking a region below the comb for the height of the stacker you want. Look for bubbles and remove them, then layer the top of the gel with water saturated butanol or, very carefully, with water. This will remove bubbles at the top of the gel and will ensure this part does not dry out. Wait for about 30 minutes for the gel to polymerize completely. (If you always use fresh ammonium persulfate, you're gel may polymerize more quickly and reliably).

While waiting mix the reagents for the stacking gel, but leave OUT the APS and TEMED until you are ready to pour this gel; stacking gels will polymerize more quickly than desired sometimes while one is trying to add combs to make wells.

When the running gel is polymerized wash out the butanol completely or your stacker may separate from the gel and you will get ugly running artifacts. Mix in the polymerizing reagents and pour the stacking gel on top of the running gel. Insert your combs trying not to get bubbles stuck underneath and allow another 30 min - 1 hour for complete polymerization.

Samples Preparation: Mix your protein 4:1 with the sample buffer (pH = 6.8). Heat your sample by 95 °C for 5 minutes.

Running Gel: Gel was clamped and both buffer chambers (pH = 8.8) were filled with gel running buffer. The sample was pipetted into the gel adjusting the volume according to the amount of protein in the sample. If you are going to stain using Coomassie, don't use much more than 5 ìg of your protein of interest to get a nicely defined band. Be sure to include a lane with molecular weight standards. Now attach your power leads and run the gel until the blue dye front reaches the bottom. I prefer to run at 250 V constant which in a four to twenty percent mini gel needs about 30 minutes total run time, but adjust to the thickness of your gel, the power supply used and the resolution desired. Remove the gel for the power supply and process further - Visualize your proteins using Coomassie Brilliant Blue, Silver stain, or any of the other protein stains. Use a carbohydrate stain for glycoproteins, or blot your gel for N-terminal sequencing or Western blotting.

RESULTS AND DISCUSSION

Chemical composition of white oats and Barley flours are shown in Table (1). Barley flour exhibited the lowest ash and fat with highest Total Carbohydrate and total protein contents, compared with those of white oats. On the other hand, white oats possessed high values of fiber and fat.

It was reported that oat germ rich in lipid and hydrolytic enzymes, so it is important in storage stability of the final products [22].Oat endosperm is rich in soluble fiber especially β-glucan as well as protein and pentosans [23]. Considerable variation exists in the dietary fibre and starch content of barley grain which results in a tremendous amount of variation in digestible energy content [24]. Moreover, barley is very good source of dietary fiber, manganese, selenium, copper, vitamin B1, chromium, phosphorus, magnesium, niacin and protein [25].

Table 1: Chemical composition (%) of white Oats and Barley flour (WW)

Composition (%)	White Oat flour	Barley flour
Total Protein	11.0	12.2
Fat	8.0	2.12
Total Carbohydrates	60.0	71.49
Fiber	8.5	6.85
Ash	4.8	3.64

Electrophoretic Characterization of Yoghurts: The patterns of electrophoresis were performed to monitor Barley flour and white oats by yoghurt starter, (*Streptococcus thermophillus, Lactobacillus delbrueckii ssp. bulgaricus*) (Figs. 1, 2, 3 and 4) at different storage periods from 0.0, 7.0 and 14 days. The electrophoresis patterns (lanes 2, 3, 4,) were characterized by the appearance of some new bands in Barley flour region. This could be attributed to the degradation of Barley flour. After 14 days of storage, it could be observed that the intensity of Barley flour band decreased compared to that of control (lane1). Although the intensity of Barley flour bands decreased, no band could be detected in the 2% Barley flour region after 7 days. This could be attributed to a loss of Barley flour fragments (lane 4, 5,6,7). The degradation in both Barley flour (Fig. 2, lane 5, 6,7) was very slight compared to white oats flour. It could be concluded that the derivatives formed were characterized in terms SDS -PAGE. It did not show any difference in band pattern i.e. no difference in mobility based on size of the proteins, but the intensity (width) of band differs when used Barley flour. White Oat flour illustrates the difference in electrophoretic migration of the soluble (supernatant) and insoluble (pellet) fractions under different concentrates of white Oat flour with three different times. In general, the intensity and numbers of bands decreased as solubility decreased in supernatants under 0 days conditions.

The SDS-PAGE technique was used to analyze the size distribution of the samples in terms of molecular weight [26]. These results are in line studied the interaction of plant phenols with whey proteins. The formation of high molecular weight fractions was documented with SDS-PAGE. Especially the derivatives of chlorogenic, caffeic, gallic acid and p-quinone showed an increase in molecular weight of β-lactoglobulin fraction from 50, 68, 75, 180 KDa [27]. The reported the ability of phenolic compounds to interact with (and even precipitate) proteins, particularly proline rich proteins such as salivary proteins and caseins [28]. The principal cohesive forces under mild conditions appear to be hydrophobic and hydrogen bonding [29].

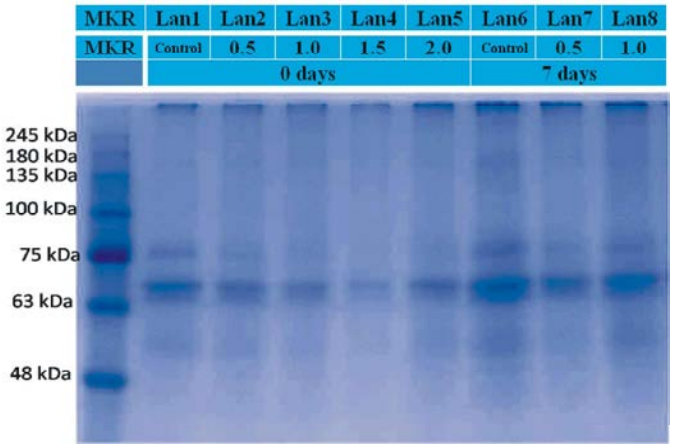

Fig. 1: SDS-PAGE of Yoghurt with Barley flour at different levels.

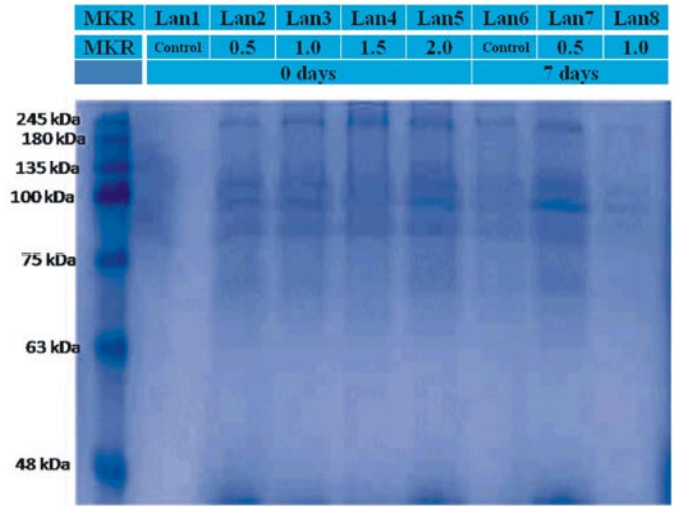

Fig. 2: SDS-PAGE of Yoghurt with white Oat flour at different levels.

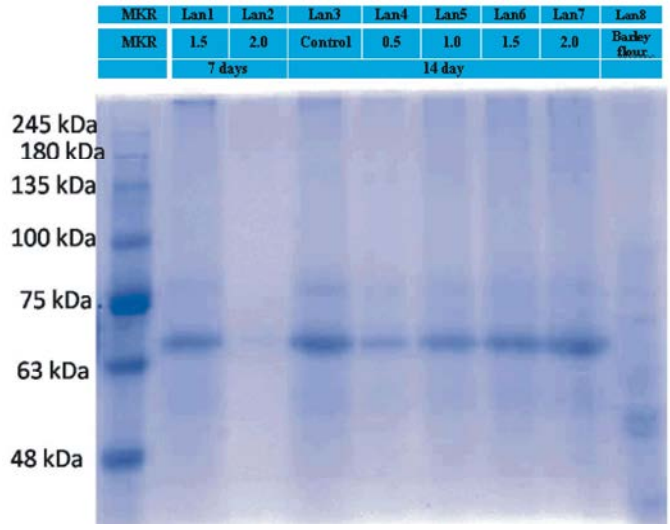

Fig. 3: SDS-PAGE of Yoghurt with Barley flour at different levels.

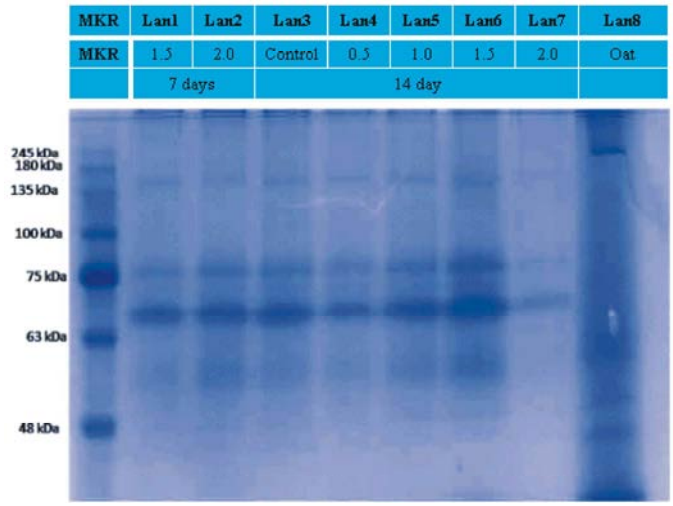

Fig. 4. SDS-PAGE of Yoghurt with white Oat flour at different levels.

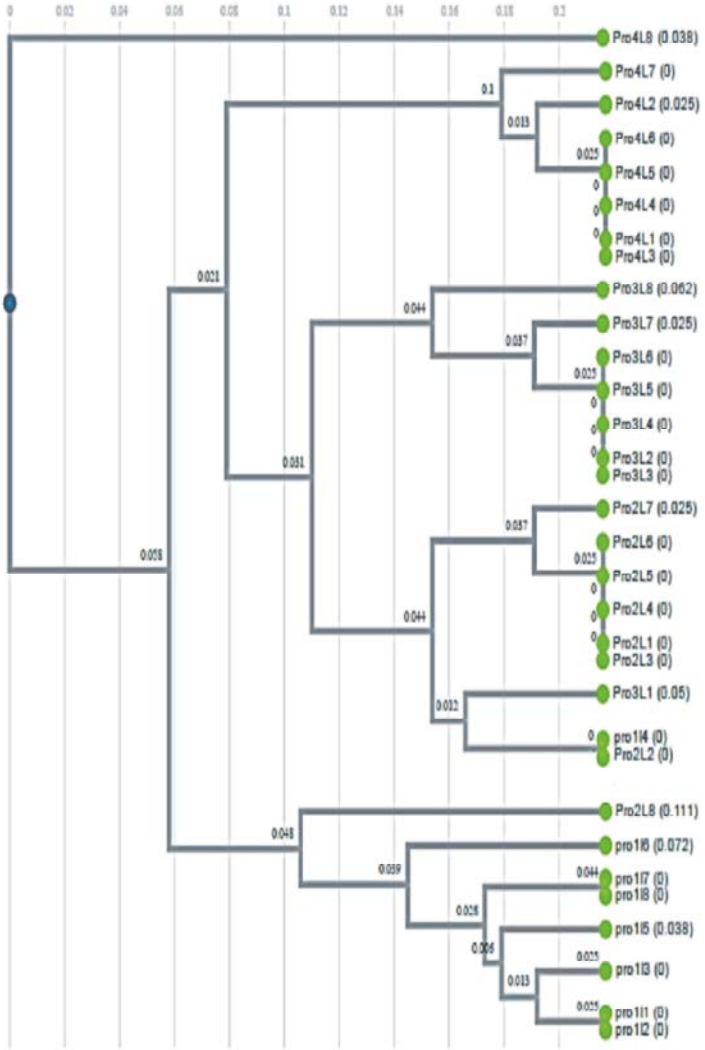

Fig. 5: Cluster analysis (UPGMA) dendrogram showing the genetic relationships between the isolates based on SDS-PAGE data of Yoghurt with Barley and white Oat flours and Jaccard's similarity coefficient.

Cluster Analysis (UPGMA) Dendrogram: A Dendrogram (from the Greek Dendron "tree" and gramma "drawing") is a diagram frequently used to illustrate the arrangement of different clusters produced by hierarchical clustering. It consists of U-shaped lines that connect data points in a hierarchical tree. The length of lines represents the distance between two data points that is connected. A dendrogram is not, however, a phylogenetic tree because it does not show evolutionary information.

Cluster analysis (UPGMA) was used to produce a dendrogram showing the genetic relationships between the isolates based on SDS-PAGE data and Jaccard's similarity coefficient.

Results Could Be Summarized as Follow:

- Pro4L8 (white oat flour): out of Group.
- Pro4L7 (white oat 2.0% at 14 days): Closely related to Pro4L2 (white oat 2.0% at 7 days) .
- Pro4L2 (white oat 2.0% at 7 days): Closely related to (Pro4L6 (white oat 1.5% at 14 days), Pro4L5 (white oat 1.0% at 14 days), Pro4L4 (white oat 0.5% at 14 days), Pro4L1 (white oat 1.5% at 7 days) and Pro4L3 (control at 14 days)).
- (Pro4L6 (white oat 1.5% at 14 days), Pro4L5 (white oat 1.0% at 14 days), Pro4L4 (white oat 0.5% at 14 days), Pro4L1 (white oat 1.5% at 7 days) and Pro4L3 (control at 14 days)). Identical to each other.
- Pro3L8 (white oat 1.0% at 7 days) : Closely related to Pro3L7(white oat 0.5% at 7 days)
- Pro3L7 (white oat 0.5% at 7 days): Closely related to (Pro3L6 (control at 7 days), Pro3L5 (white oat 2.0% at 0 time) , Pro3L4 (white oat 1.5% at 0 time), Pro3L2 (white oat 0.5% at 0 time) and Pro3L3(white oat 1.0% at 0 time)).
- (Pro3L6 (control at 7 days), Pro3L5 (white oat 2.0% at 0 time) , Pro3L4 (white oat 1.5% at 0 time), Pro3L2 (white oat 0.5% at 0 time) and Pro3L3(white oat 1.0% at 0 time)) Identical to each other.
- Pro2L7 (Barley 2.0% at 14 days) closely related to Pro2L6 (Barley 1.5% at 14 days).
- (Pro2L6 (Barley 1.5% at 14 days), Pro2L5 (Barley 1.0% at 14 days), Pro2L4 (Barley 0.5% at 14 days), Pro2L1 (Barley 1.5% at 7 days) and Pro2L3 (control at 14 days)) Identical to each other.
- Pro3L1 (control at 0 time) closely related to Pro1L4 (Barley 1.5% at 0 time).
- (Pro1L4 (Barley 1.5% at 0 time) and Pro2L2 (Barley 2.0% at 7 days)) Identical to each other.
- Pro2L8 (Barley flour) closely related to Pro1L6 (control at 7 days).
- Pro1L6 (Barley 1.5% at 0 time). Closely related to Pro1L7 (Barley 0.5% at 7 days).
- (Pro1L7 (Barley 0.5% at 7 days). and Pro1L8 (Barley 1.0% at 7 days).) Identical to each other.
- Pro1L5 (Barley 2.0% at 0 time) closely related to Pro1L3 (Barley 1.0% at 0 time).
- Pro1L3 (Barley 1.0% at 0 time) closely related to Pro1L1 (control at 0 time).
- (Pro1L1 (control at 0 time) and Pro1L2 (Barley 0.5% at 0 time)) Identical to each other.

CONCLUSIONS

From the obtained results addition of barley or white oat flours at level up to 1.0% to yoghurt could be recommended. These additives increased Total Solids and Total Protein contents of resultant yoghurt and improved the organoleptic properties of resultant yoghurt.

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