**Table 2** Repeatability and recovery of the proposed analytical method applied to five standards of DG. (*PP* Dipalmitolglycerol, *SS* distearilglycerol, *PS* palmitil-stearilglycerol)

| Standard | $\bar{X}$ | s(σ) | CV% | Recovery % |
|---|---|---|---|---|
| 1,2-PP (mg) | 0.28 | 0.020 | 7.2 | 81.0 |
| 1,2-SS (mg) | 0.28 | 0.034 | 11.9 | 97.8 |
| 1,3-PP (mg) | 0.28 | 0.035 | 12.5 | 76.8 |
| 1,3-SS (mg) | 0.25 | 0.023 | 9.3 | 73.1 |
| 1,3-PS (mg) | 0.29 | 0.040 | 13.9 | 82.0 |

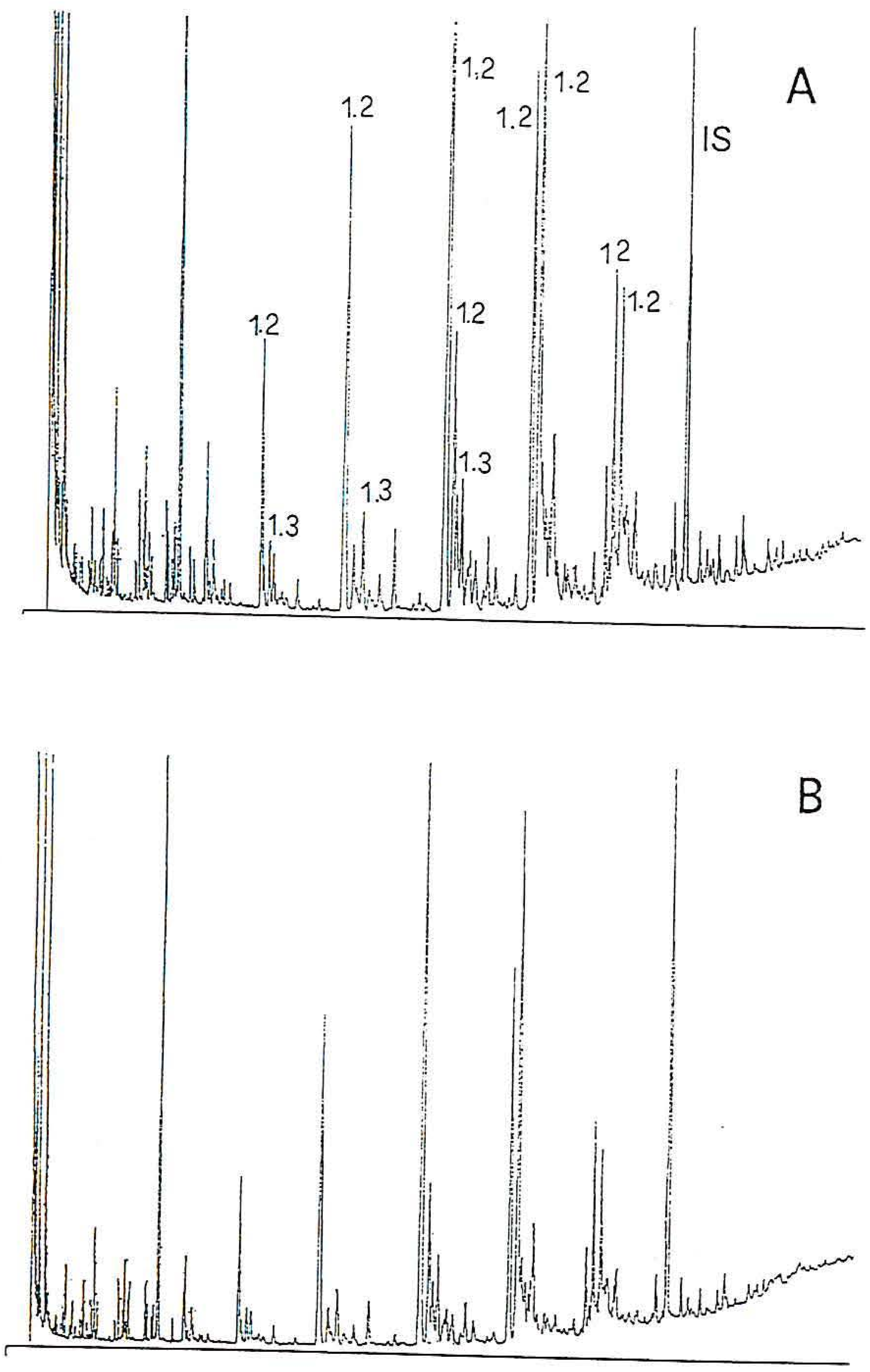


**Fig. 3 A** GLC trace of the diglycerides fraction eluted from a new SPE diol cartridge. **B** GLC trace of the diglycerides fraction eluted from a re-used SPE diol cartridge (five elutions)

application of two co-ordinated chromatography techniques, each of which introduces a given error, and taking account of the fact that the calculated response factor is an average factor, derived from the sum of the areas of all the peaks ascribable to both the 1,2-forms and the 1,3-forms.

For all the validations of the fat extracted from the cheese, only those diglycerides that eluted after cholesterol were considered, because of the presence of short-chain fatty acids. In fact, in theory monoglycerides of long chain acids can elute at the same time as diglycerides of short chain acids; therefore, it was preferred not to include peaks that were difficult to interpret in the analysis. For this reason, the validation trials of recovery were carried out using standards in a matrix of butter triglycerides.

The coefficients of variation recorded for the standards agreed with those obtained for the cheese fat (Table 2); the recovery values were quite high, confirming the validity of the method.

Lastly, the possibility of re-using the SPE columns was evaluated. After the elution of a sample, the columns were re-treated, as described in Materials and methods, and re-used up to four times. As is shown in Fig. 3, no appreciable deterioration in appearance of the chromatography trace is evidenced; the different dimensions are due exclusively to the quantity injected, slightly less in the latter case. The fact remains that, after being used four times, the diol phase column is not altered in its capacity to prevent isomerization.

In conclusion, this study has demonstrated the following. First, that it is possible to separate the diacylglycerols prior to analysis, in such a way as to obtain a solution for analysis by GLC that is almost free of substances that may cause interference, in particular those that boil at high temperatures, as long as isomerization is avoided. Second, the aminopropyl phase, often proposed for this type of separation in place of the silica phase because it is less affected by traces of water, gives rise to a high isomerization rate. Third, the diol phase allows complete elution of the diacylglycerols with lower volumes and in the total absence of isomerization. Fourth, the repeatability of the proposed method is good, considering the complexity of the operations. Fifth, analogously, the recovery characteristics of the proposed method are more than adequate.

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ORIGINAL PAPER

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# Fortification of sweetened plain yogurt with insoluble dietary fiber

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**Abstract** Seven types of insoluble dietary fiber from five different sources (soy, rice, oat, corn and sugar beet) were used to fortify sweetened plain yogurt. Fiber addition caused an acceleration in the acidification rate of the experimental group yogurts, and most of the fortified yogurts also showed increases in their apparent viscosity. Soy I and sugar beet fibers caused a significant decrease in viscosity due to partial syneresis. In general, fiber addition led to lower overall flavor and texture scores. A grainy flavor and a gritty texture were intense in all fiber-fortified yogurts, except in those made with oat fiber. Oat II fiber gave the best results; differences with controls in terms of flavor quality scores not being statistically significant. The evolution of organic acids during the fermentation and cold storage of control and oat-II-fiber-fortified yogurts showed a similar pattern; only acetic and propionic acids were found in significantly higher amounts in the fiber-fortified product.

**Key words** Dietary fiber · yogurt · Fermentation · Organic acids · Sensory assesment

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## Introduction

Today, consumers are concerned about the nutritional aspects of the food they eat. Fermented milk products have been recognized by nutritionists as being beneficial to human health [1]. Fermented milk products contain significant levels of organic acids, among other substances, which may help in the cure of illnesses such as diarrhea [2] and hypercholesterolemia [3]. Dietary fiber plays a major role in the prevention of constipation, hemorrhoids, hypercholesterolemia and colon cancer [4]. Labell [5] recommends a daily intake of 25–30 g of dietary fiber for adults. It is therefore reasonable to expect a steady increase in the consumption of healthy, fiber-rich foods in the future.

Increasing the fiber content of a product offers a challenge to both product developers and ingredient suppliers. They need to learn what sources are available, into which existing products fiber can be incorporated and what new products can be developed.

Chemical compounds from the fiber may interact with food components during processing. Interactions between ingredients may lead to changes in nutrient bioavailability and the texture and flavor of the final product [6]. Studies of the effect of fiber addition to dairy products are scarce. Most fiber addition studies have been carried out in connection with the improvement of ice cream texture through the addition of stabilizers and emulsifiers (pectins, Arabic gum, xhantan, alginates) [7]. Only a few studies of the development of fiber-fortified and flavored milk drinks have been carried out [8].

We have compared seven types of insoluble dietary fiber from five different origins for their application in the development of a fiber-fortified fermented milk. An addition level of 1.3% dietary fiber was selected according to regulations in the USA for fiber-fortified products. This amount would account for approximately 10% of the recommended daily intake of fiber, if 200 ml of yogurt was consumed daily. The evolution of

**Table 1** Used fibers: characteristics, origin, commerical name and supplier. (*TDF* % total dietary fiber, *SDF* % soluble dietary fiber, *IDF* % insoluble dietary fiber, *WHC* water-holding capacity)

| Fiber | Commercial name | TDF (%) | SDF (%) | IDF (%) | WHC[a] (%) | Supplier |
|---|---|---|---|---|---|---|
| Corn | Nu 20085 Ultrafine corn bran dietary fiber | 88 | 2 | 86 | 200 | Lanhoff Grain, Danville, Ill., USA |
| Oat I | Snowite Fine oat fiber | 90 | 2 | 88 | 310 | Canadian Harvest, Cambridge, Minn., USA |
| Oat II | Better Basics 782 Advanced oat fiber | 97 | 0 | 97 | 630 | Williamson Food Ing, Louisville, Ky., USA |
| Soy I | Tu 20070 Ultrafine soy dietary fiber | 75 | 10 | 65 | 200 | Lanhoff Grain, Danville, Ill., USA |
| Soy II | Fibrim 2000 Soy fiber | 75 | 65 | 10 | – | Protein Technologies Int, St. Louis, Mo., USA |
| Sugar beet | Fibrex 585 Sugar beet fiber | 74 | 24 | 50 | 550 | Delta Fiber Foods Minneapolis, Minn., USA |
| Rice | Protex 20-S Rice fiber | – | 22 | 20 | 65 | Riviana Foods, Houston, Tex., USA |

[a] From Angelino [8]

fermentation was followed by monitoring pH and organic acid production. Organic acids are important components for the sensory properties of fermented foods, and as indicators of bacterial activity [9–11]. Sensory analysis of the final product was also performed.

## Materials and methods

*Dietary fibers.* Seven fibers were evaluated. Table 1 details their origin, characteristics and supplier, as well as the label we have used for them throughout the study.

*Yogurts.* Yogurts were manufactured, using 1000-ml stainless steel containers, from reconstituted skim milk and pasteurized, homogenized whole milk standardized to 12% solids-non-fat and 1% fat. All yogurts were supplemented with 4% sucrose. Fiber was added at 1.32%. The mix was homogenized in a Stomacher (Tekmar 400; Tekmar, Cincinnati, Ohio, USA) heated at 85 °C for 30 min, cooled to 42 °C, inoculated with 0.04% of a concentrated frozen starter culture consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (1:1) (CH-3; Christian Hansens, Milwaukee, Wis., USA) and incubated at 42 °C to a final pH of 4.4 (7–8 h). The coagulum was then broken and the stirred yogurt was aseptically transferred to plastic cups and stored at 4 °C for 4 weeks. Triplicate trials were conducted.

Samples for pH and organic acid analyses were taken, under sterile conditions, in whirl-pak (Nasco, Fort Atkinson, WI) sampling bags at 1-h intervals during fermentation and after 4 weeks of storage at 4 °C. pH was measured with a SA250 pH meter (Orion, Boston, Mass., USA).

*Apparent viscosity.* Apparent viscosity of the sample was measured at a temperature of 7 °C, with the sample in a 250-ml beaker, using a Helipath adapter for thixotropic fluids. Samples were tested using a T-spindle (T-E size) coupled to an LVTD Digital viscometer (Brookfield Engineering, Stoughton, Mass., USA). The viscometer was set at 1.5 rpm. Yogurt was gently stirred for 20 s before analysis and triplicate measurements were conducted.

*HPLC of organic acids.* Orotic, citric, pyruvic, lactic, uric, formic, acetic, propionic, butyric and hippuric acids were extracted with 0.01N $H_2SO_4$ and separated in an ion-exchange column (AMINEX HPX-87H 300 × 7.8 mm, Bio-Rad, Richmond, Calif., USA). Chromatographic conditions and HPLC apparatus have been described in a previous work [12]. Quantification was carried out by the external standard method. Quantification of pyruvic acid was difficult because sucrose eluted just after the pyruvic peak. For this reason a standard curve was constructed using organic acid solutions supplemented with 4% sucrose. The pyruvic acid peak was tangent skimmed from the sucrose peak and quantified by height. Duplicate extractions and injections were conducted for each sample.

*Sensory analysis.* A four-member expert panel, trained in the examination of dairy products, was used to evaluate the fiber-fortified yogurts. Panelists scored the samples for overall flavor quality (10-point scale) and appearance and mouthfeel quality (5-point scale). Sample defects and attributes such as acid, sweet, bitter, acetaldehyde, grainy, off-flavor, ropy and gritty texture, whey syneresis and lumpiness were scored using a 7-point intensity scale (1 = not detectable; 7 = extreme).

*Statistical analysis.* One-way analysis of variance was conducted by using the SPSS PC + 4.0 software (SPSS, Chicago, Ill., USA). The Duncan test was applied for means comparison.

## Results and discussion

Fiber addition caused an acceleration in the acidification rate of experimental group yogurts compared with controls (Fig. 1). This acceleration was statistically significant ($P < 0.05$) in the early fermentation phase of yogurts made with soy I and II, sugar beet and rice fibers. The difference among treatments decreased with time, but controls maintained a slower acidification rate during the whole fermentation process. Some fibers could have supplied nutrients or growth stimulant factors for the starter culture. Sugar beet fiber addition caused a milk pH decrease prior to inocula-

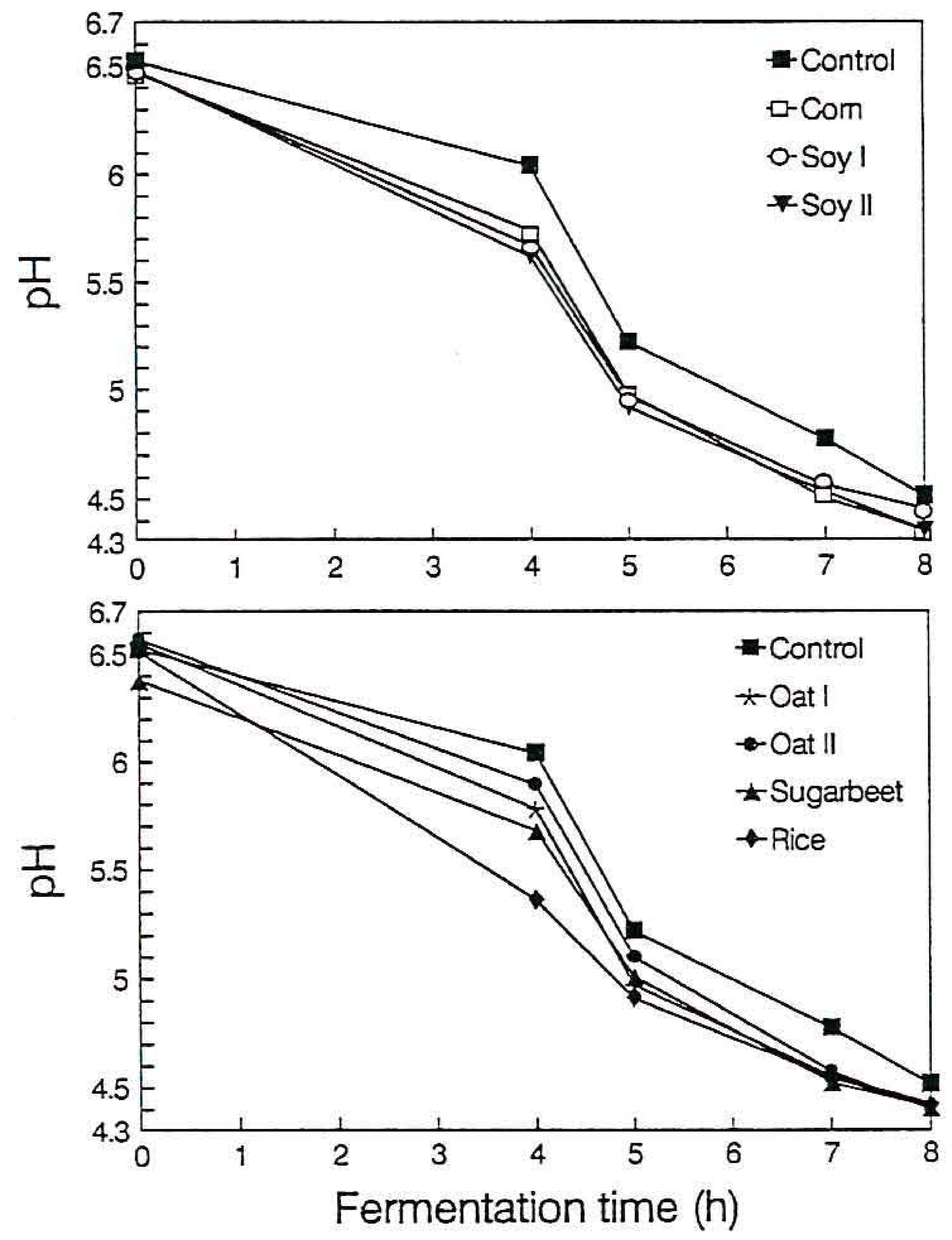


**Fig. 1** Evolution of mean pH during fermentation of sweetened plain yogurt supplemented with 1.32% of 7 different dietary fibers. Control (■); yogurts with corn (□), soy I (○), soy II (▼), oat I (*), oat II (●), sugar beet (▲) and rice (◆) fibers

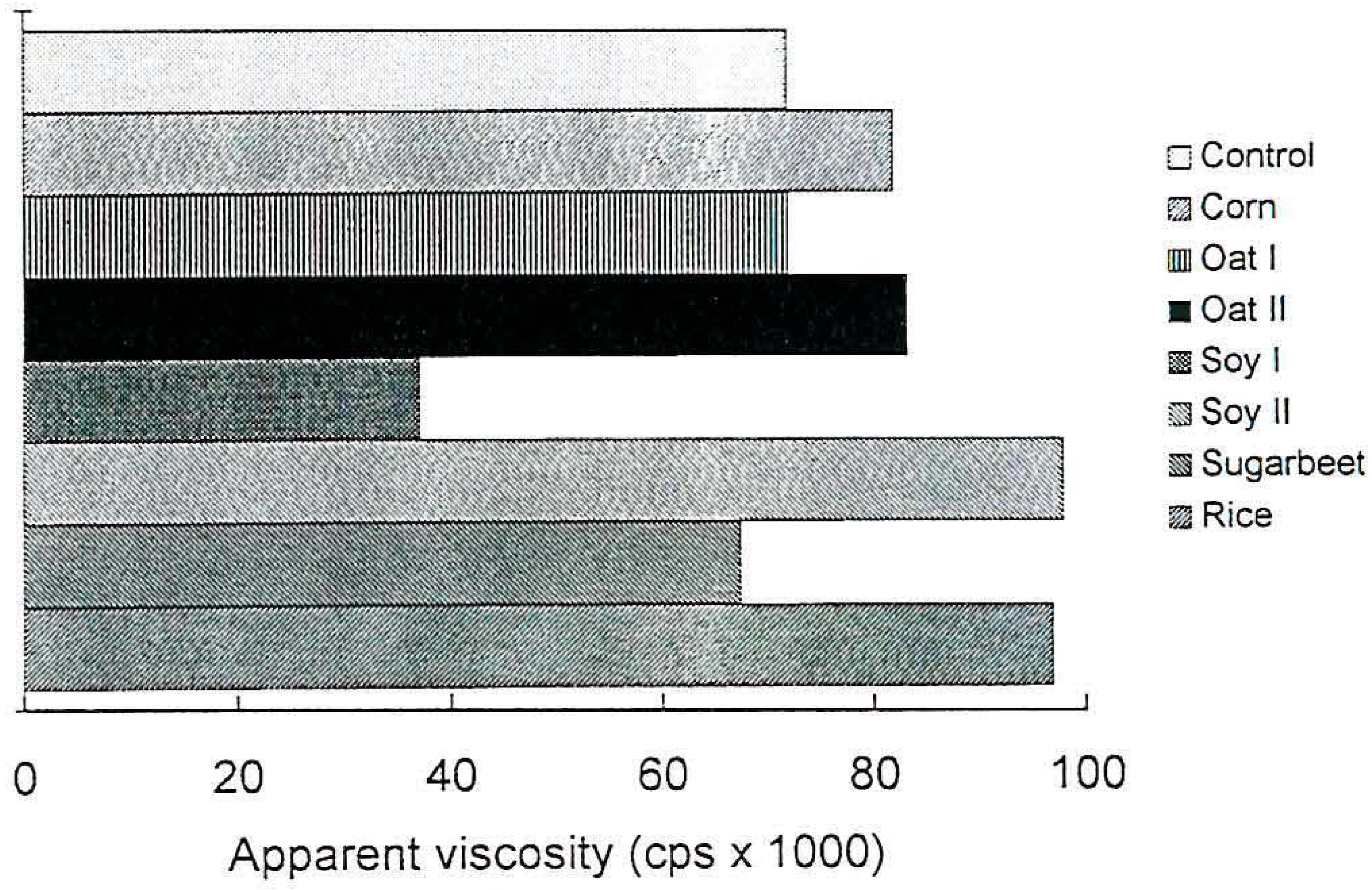


**Fig. 2** Mean apparent viscosity of yogurts supplemented with 1.32% of dietary fibers

tion, possibly due to acid components of the fiber product.

Most of the fiber treatments increased the apparent viscosity of the final product but differences were not significant (Fig. 2). The viscosity increase has been attributed to interactions between the exogenous hydrocolloids and dairy proteins [13, 14]. Soy I and sugar beet fiber addition caused a decrease in viscosity. The viscosity decrease in yogurts with added soy I was statistically significant ($P < 0.05$). This decrease can be attributed to partial whey syneresis (Table 2). In these yogurts a clear precipitation of caseins with the fiber particles was produced. The body and texture evaluation of these samples showed definite aggregates which were organoleptically detectable and partially separated from whey [15, 16]. A relationship was not found to occur between the water-holding capacity or solubility of the fibers (Table 1) and the viscosity increase in the fiber-fortified yogurts.

The appearance of the experimental group yogurts was significantly ($P < 0.05$) affected by the addition of dietary fibers (Table 2). Soy I, sugar beet and rice fibers showed definite lumpiness and appreciable color change due to whey syneresis.

Yogurts made with oat fiber were not significantly different ($P > 0.05$) from the controls and were deemed to be commercially acceptable (score of 7 or higher). Corn-fiber-fortified samples received slightly lower overall scores than the oat-fiber-fortified samples. However, in general, fiber addition led to a significant decrease ($P < 0.05$) of the overall flavor score for

**Table 2** Mean values[1] for the sensory evaluation of fiber-fortified yogurts

| | Control | Corn | Oat I | Oat II | Soy I | Soy II | Sugar beet | Rice |
|---|---|---|---|---|---|---|---|---|
| Flavor[2] | 8.2$^{a}$ | 6.8$^{abc}$ | 7.1$^{ab}$ | 7.7$^{ab}$ | 4.2$^{d}$ | 6.6$^{bc}$ | 6.1$^{bc}$ | 5.3$^{cd}$ |
| Texture[3] | 4.7$^{a}$ | 3.3$^{ab}$ | 3.4$^{ab}$ | 3.8$^{ab}$ | 1.3$^{c}$ | 3.8$^{ab}$ | 3.0$^{b}$ | 2.7$^{b}$ |
| Appearance[3] | 4.8$^{a}$ | 3.6$^{bc}$ | 4.0$^{abc}$ | 4.5$^{ab}$ | 2.6$^{dc}$ | 4.0$^{abc}$ | 2.3$^{d}$ | 3.0$^{dc}$ |
| Acetaldehyde[4] | 5.0$^{a}$ | 3.8$^{a}$ | 3.8$^{a}$ | 4.7$^{a}$ | 1.0$^{b}$ | 4.8$^{a}$ | 4.0$^{a}$ | 1.0$^{b}$ |
| Acid[4] | 4.3$^{b}$ | 5.0$^{b}$ | 4.5$^{b}$ | 4.5$^{b}$ | 3.5$^{b}$ | 3.8$^{b}$ | 6.0$^{a}$ | 3.5$^{b}$ |
| Grainy[4] | 1.0$^{c}$ | 3.4$^{b}$ | 3.0$^{cd}$ | 2.1$^{d}$ | 5.6$^{a}$ | 4.7$^{ab}$ | 5.1$^{ab}$ | 6.0$^{a}$ |
| Off-flavor[4] | – | – | – | – | 6.0 | – | 4.0 | 4.7 |
| Ropy[4] | 1.2 | 2.8 | 2.3 | 2.1 | 1.5 | 2.7 | 3.2 | 2.3 |
| Gritty[4] | 1.0$^{d}$ | 3.2$^{bc}$ | 3.5$^{bc}$ | 2.2$^{c}$ | 5.7$^{ab}$ | 2.3$^{c}$ | 3.8$^{abc}$ | 4.5$^{ab}$ |
| Whey syneresis | No | No | No | No | Yes | No | Slight | No |
| Color | White | Yellowish | White | White | Brownish | White | Brownish | Marfil |

[1] Mean values from 3 trials
[2] Overall flavor quality on a 10-point scale (1 = poorest quality; 10 = best quality)
[3] Overall texture and appearance quality on a 5-point scale (1 = poorest quality; 5 = best quality)
[4] Attributes on a 7-point intensity scale (1 = not detected; 7 = extremely high)
[abc] Means within the *same row* sharing *superscripts* do not differ significantly ($P > 0.05$)

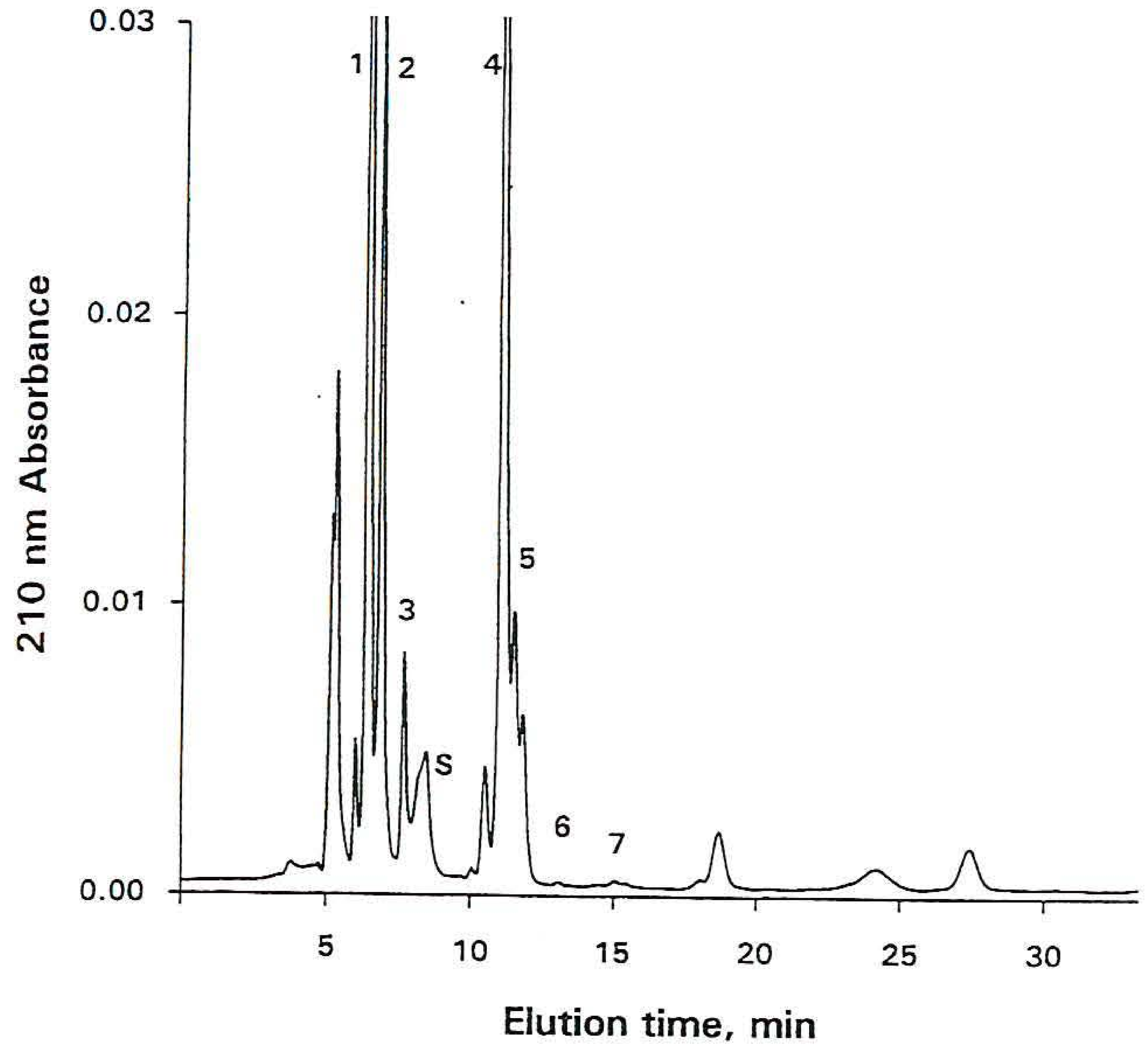


**Fig. 3** Ion-exchange HPLC chromatogram corresponding to a 0.01N $H_2SO_4$ extract of sweetened plain yogurt made with addition of 1.32% oat II fiber. Organic acids: (*1*) orotic; (*2*) citric; (*3*) pyruvic; (*4*) lactic; (*5*) uric; (*6*) acetic; (*7*) propionic, (*S*) saccharose

yogurts (Table 2). In most cases underscored samples also showed a variable depression of their characteristic acetaldehyde and acid flavors (yogurts with oat I, soy I and II and rice fibers). Depressions in the basic taste sensations (sweet, acid, salty, and bitter) have been reported to occur following hydrocolloid addition, and headspace GC has shown changes in the volatility of some compounds in sugar/hydrocolloid mixtures [17]. Sugar-beet-fiber-fortified yogurts were found to be significantly more acidic than other fiber-treated yogurts ($P < 0.05$) but not compared to controls. The grainy flavor was significantly more intense ($P < 0.05$) in all fiber-fortified yogurts, except those fortified with oat II, as compared with controls. The most intense grainy flavors were detected in yogurts containing soy, sugar beet and rice fibers. The detection of off-flavors and the lack of a fresh aroma led to lower scores for yogurts fortified with soy I, sugar beet and rice fibers.

The overall texture quality was the sensory parameter most affected by fortification with dietary fiber. These low scores were primarily due to the grittiness effect (Table 2), which was more intense in all fiber-fortified yogurts as compared with controls. Yogurts made with soy I fiber received the highest gritty texture values, while those made with oat II fiber had the lowest. Although samples varied in their apparent viscosity measurements (Fig. 2), there were no significant differences in their ropy-texture characteristics with the exception of atypical yogurts made with soy I fiber. Previous work in our laboratory with fluid products has shown similar results. The oat I and II fibers performed best in fluid products. Significant improvements in fluid products were also achieved by manipulating the stabilization system. This previous work also showed that the effect of the stabilizing systems is dependent on fiber type. Alteration in the yogurt stabilizing system may help to improve overall quality [8].

The evolution of organic acids during fermentation was only studied in control yogurts and those fortified with oat II fiber (Better Basics), because they were the products with the best sensory characteristics. Figure 3 shows the HPLC chromatogram corresponding to an oat-II-fiber-fortified yogurt extract. Table 3 shows the mean values for the organic acid concentration in yogurts during fermentation and after a 4-week cold-storage period. The citric acid concentration (2184 μg/g on average) did not change during fermentation or cold storage and was not affected by the addition of oat fiber (data not shown). Neither butyric nor formic acids were detected during the whole process. Hippuric acid disappeared completely after 4 h of fermentation, probably having been converted to benzoic acid by lactic acid bacteria [18]. The orotic acid concentration decreased and lactic, acetic and propionic acid concentrations increased significantly ($P < 0.01$) throughout milk fermentation and cold storage. Similar evolution patterns of organic acids in non-sweetened plain yogurt have been observed previously [12]. Orotic acid is a nucleic acid precursor, a growth factor for lactic acid bacteria [19] and is actively consumed through milk fermentation [10, 20]. Higher hippuric acid and orotic acid consumption rates were observed to occur in the fiber-fortified yogurts, although differences with controls were not significant ($P > 0.05$). Fiber addition did not significantly affect lactic acid production by the starter cultures although, at the end of fermentation and after 4 weeks of cold storage, a higher concentration of lactic acid in the fiber-fortified yogurts with similar pH values was observed. This could have been due to a fiber buffer capacity. Pyruvic acid levels increased to a maximum during the first 6 h of fermentation, decreasing afterwards, probably as it was being utilized in diverse metabolic pathways [12]. Almost significantly lower pyruvic acid levels ($P = 0.09$) and significantly higher acetic acid levels ($P < 0.01$) were observed to occur during fermentation in fiber-fortified yogurts as compared with controls. Almost significantly higher propionic acid concentrations ($P = 0.1$) were detected for fiber-fortified yogurts as well. These changes could be related to increased consumption rates for orotic and hippuric acids and higher lactic acid production by the starter in the fiber-fortified yogurts. The presence of oat fiber during fermentation could be stimulating starter growth, although differences were not statistically significant. During refrigeration, the pyruvic acid concentration in control and experimental group yogurts reached similar values.

Fortification of yogurt with oat fiber produced the highest overall quality products. Fiber addition resulted in a significant lowering of overall texture quality. The HPLC study of organic acids showed that concentrations of acetic and propionic acids increased at a higher rate in fiber-fortified yogurts than in controls. The acetic acid increase was probably due to the acetic acid content of the fiber itself, since it was observed to occur in the milk with added fiber. This slight increase was, however, not detected by the panelists.

When a new fiber-fortified product is developed, one must be willing to sacrifice some characteristics of the original product (flavor, texture, appearance), taking into account the healthy effect of the new product. Oat fiber addition permitted the development of a good fermented product, with no significant decrease in flavor quality and only a slight decrease in texture quality. Oat fiber addition to sweetened plain yogurt may prove to be an efficient method of increasing dietary fiber intake (around 10% of the recommended daily intake with a yogurt consumption of 200 ml) in a pleasant way.

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**Table 3** Mean values[1] for the organic acids content during fermentation of oat-fiber-fortified yogurts

| Fermentation time (h) | Organic acid | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Orotic | | Pyruvic | | Lactic | | Acetic | | Propionic | | Hippuric | |
| | Control | Oat fiber | Control | Oat fiber | Control | Oat fiber | Control | Oat fiber | Control | Oat fiber | Control | Oat fiber |
| 0 | 117.7 | 117.5 | 7.27 | 5.46 | – | – | 3.08 | 13.97 | – | – | 21.28 | 21.08 |
| 4 | 114.3 | 113.3 | 27.54 | 19.05 | 2200 | 2180 | 15.65 | 27.16 | 3.87 | 7.04 | 2.4 | 1.19 |
| 6 | 103.3 | 102.4 | 55.23 | 50.1 | 6221 | 6159 | 28.83 | 37.63 | 25.01 | 25.24 | 0 | 0 |
| 8 | 88.74 | 84.68 | 41.05 | 29.78 | 8613 | 9455 | 41.06 | 50.26 | 29.91 | 49.65 | 0 | 0 |
| 4-week Refrigeration | 82.95 | 83.89 | 22.34 | 26.69 | 11094 | 11665 | 59.57 | 66.62 | 47.35 | 56.73 | 0 | 0 |
| Mean | 101.4[a] | 100.4[a] | 30.68[a*] | 26.21[a*] | 5626[a] | 5886[a] | 29.63[b] | 41.92[a] | 29.75[a*] | 34.66[a*] | | |

[1] Mean values from two replicates expressed in µg/g yogurt
[ab] Means for each acid followed by the *same superscript* do not differ significantly ($P > 0.05$)
[a*] Means differ almost significanlty ($P \leq 0.1$)

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