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EFFECT OF ADDITION OF WHEY PROTEIN AGGREGATES OBTAINED BY SINGLE AND DOUBLE HEATING METHOD ON THE RHEOLOGICAL PROPERTIES OF SET YOGHURTS

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The effect of addition of single (shWPI) and double-heated whey proteins polymers (dhWPI) to set yoghurts and its influence on the rheological properties was investigated. The hardness of set yoghurt was investigated by TA-XT2i Texture Analyser. The yoghurt fermentation process was monitored by the oscillatory rheometer for 4 h at 43°C and next cooling to 4°C and stored for 3 h. The gelation of yoghurt started up more quickly for shWPI and dhWPI fortified yoghurts in comparison to the control samples. Size–exclusion HPLC indicated that native WPI formed soluble aggregates after heat-treatment. The heating time in polymerisation process affected the content of whey protein polymers/aggregates in the solution. The use of WPI solutions heat treated longer led to the improvement of the rheological properties of set yoghurts, which resulted in the increasing of their hardness. Yoghurts fortified by the 1% of dhWPI and shWPI addition, heated for the same period of time during polymerisation process exhibited similar rheological properties. This indicates that heating time is the main, determinant parameter of the polymerisation process.

INTRODUCTION

Physical properties of set yoghurt are one of many quality parameters. Two major defects in set yoghurts are low firmness and a high level of syneresis. One of the common practices in yoghurt production process is its fortification with dried dairy ingredients [Tamime & Robinson, 1999]. Several studies have focused on the possibility of using whey proteins in yoghurt making. Whey proteins were added to yoghurts to increase total solid content of milk in order to provide better consistency, texture and creaminess to the product [Gonzalez-Martinez *et al.*, 2002]. Whey protein powder addition caused an increase in viscosity and a decrease in the syneresis [Tamime & Robinson, 1999].

Mleko & Foegeding [2000] obtained whey protein aggregates/polymers exhibiting high viscosity which can successfully replace polysaccharides in the dairy technology. Glibowski *et al.* [2006] compared rheological properties of whey protein solutions obtained by single or double heating method. Double-heated dispersions gelled faster at lower protein and calcium ion concentrations than single-heated dispersions.

The desserts obtained with double-heated whey protein polymers exhibited better rheological properties in comparison to milk desserts with carrageenan and starch addition [Mleko & Gustaw, 2000]. A significant increase in apparent viscosity and firmness of set yoghurt fortified by addition of double-heated whey protein polymers was observed. In the case of yoghurts with WPI and SMP these two parameters decreased considerably [Gustaw *et al.*, 2006].

The aim of the present work is to study the effect of addition of whey protein polymers obtained by single heating at different time to the set yoghurts. Rheological properties and microstructure of yoghurt obtained by addition of single heated and double heated whey protein polymers were compared.

MATERIALS AND METHODS

Materials. Whey protein isolate (WPI) was obtained from Davisco Foods International (Le Sueur, Mn, USA), skim milk powder (SMP) from Biomlek (Chelm, Poland), whole milk powder from OSM Krasnystaw (Krasnystaw, Poland) and YC--X11 Yo-Flex Thermophilic Lactic Culture type Yoghurt CHR was obtained from Chr. Hansen (Poland). Protein content was determined with macro Kjeldahl method [AOAC, 1984].

Heat polymerisation of WPI. Single-heated whey protein isolate (shWPI) was obtained by heating whey protein dispersions (4% w/w protein) at 80°C for 5, 15, 30, 45 and 60 min at pH 7.0. Double-heated whey protein isolate (dhWPI) was obtained by heating it at 80°C (pH 8.0). Afterwards protein solutions were cooled down to 20°C and their pH was adjusted to 7.0. Then solutions were heated again, keeping exactly the same conditions as in the first heating process.

Yoghurt preparation. The whole milk powder was reconstituted at 30°C with moderate mixing using a magnetic stirrer. After milk protein powders addition, samples were homogenized (2000 rpm, 2 min) using a H 500 homogeniser (Pol-Eko, Wodzislaw Slaski, Poland). The addition of SMP,

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shWPI and dhWPI at concentrations of 1, 2 and 3% provided product fortification. The mixtures were placed in glass jars and heated at 85°C for 30 min. Afterwards they were left to cool in order to reach the incubation temperature (42-43°C). Then the samples were inoculated with YC-X11 Yo-Flex thermophilic lactic culture at 43°C and fermented until pH 4.7 was reached. The yoghurts were cooled down and refrigerated at 4°C for 24 h before rheological evaluation.

Hardness measurements of set yoghurt. Texture analyses were performed by penetration at the crosshead speed of 1 mm/s, using a TA-XT2i texture analyser (Stable Microsystems, Goaldming, UK) equipped with a cylindrical probe (1 cm diameter). Hardness was determined at 70% deformation of set yoghurt gel.

Rheological measurements. Dynamic oscillatory measurements were performed on the yoghurt samples to monitor the fermentation process for 4 h at 43°C and then decreasing the temperature to 4°C (1°C/min). An RS 300 rheometer (ThermoHaake, Karlsruhe, Germany) with a concentric cylinder rotating bob and fixed cup measuring cell was used. Measurements were carried out at an oscillation frequency of 0.1 Hz and strain amplitude of 0.01.

Size exclusion chromatography. The effect of polymerisation method and heating time on the quantity of whey protein aggregates was determined by size exclusion chromatography. SE-HPLC was performed using a Gilson HPLC system (Gilson, Middleton, USA), mounted with a TSK-Gel -G3000$_{SWXL}$ column and TSK-gel guar column (Tosoh Bioscience, Stuttgart, Germany), as described by Ju *et al.* [1997]. Samples (control and denaturated shWPI and dhWPI) were diluted at a ratio of 1:19 with deionised water and filtered through a 0.45 μm filter (Millipore, Billerica, USA), and 20 μL of sample were injected onto the column.

RESULTS AND DISCUSSION

Heat denaturation of whey protein isolate

The elution profiles of UV absorption at 280 nm of WPI, shWPI and dhWPI are shown in Figure 1. The chromatogram of original WPI solution showed peaks of native whey proteins, including β-lactoglobulin (β-lg) and α–lactoalbumin (α-la). They were eluted between 16-19 min. The elution profiles of shWPI and dhWPI showed a single major peak eluting at 8.7 min in the case of shWPI and 9.3 min for dhWPI, respectively. This peak represented soluble aggregates [Ju *et al.*, 1997]. These aggregates probably were co-aggregates of different whey proteins [Ju & Kilara, 1998]. The peak obtained in the case of shWPI was a broader and higher in comparison to the shWPI peak. This indicates that during single heating much more whey protein polymers/aggregates were formed in comparison to double heating polymerisation.

The chromatograms of single-heated WPI aggregates obtained at different heating times (from 5 to 45 min) are shown in Figure 2. The elongation of heating time increased the number of aggregates and decreased peak's area represented by β-lg and α-la. β-lg, α–la and bovine serum albumin (BSA) interact with each of the other two proteins and form aggregates or gels by disulfide bonds and hydrophophobic interactions [Dalgleish *et al.*, 1997].

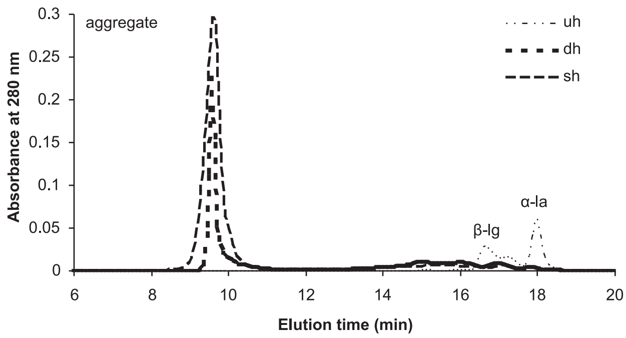

FIGURE 1. Size exclusion HPLC of unheated WPI (uh), single-heated (sh) and double-heated (dh) WPI solutions heated at 80°C for 60 min.

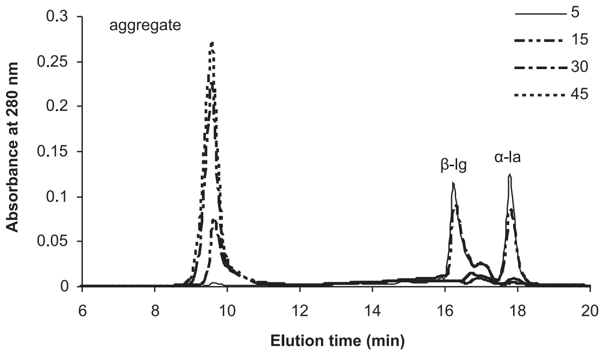

FIGURE 2. Effect of heating time (5, 15, 30 and 45 min) on WPI aggregation. WPI solutions were heated at 80°C.

Monitoring of the fermentation process

The effect of heating time on WPI polymerisation and fermentation process of yoghurts fortified by the addition of polymerised WPI is shown in Figure 3. The gelation of a yoghurt sample obtained without milk powder addition started at 43°C after 130 min of fermentation. The time of single-heated WPI polymerisation had a significant effect on the gelation of yoghurts. The increase of polymerisation time from 15 to 60 min decreased the gelation time of yoghurt from 123 to 110 min, respectively. The addition of dhWPI decreased fermentation time below 120 min; similar results were obtained in the case of sh60WPI. Gustaw *et al.* [2006] claimed that addition of WPI aggregates caused a decrease in gelation time of yoghurts. Lucey *et al.* [1999] observed that whey protein addition to milk and the subsequent heat treatment resulted in the shortening of the gelation time. However, addition of WPC to milk after heating caused an increase of this value. After fermentation, when the temperature decreased to 4°C, the highest values of storage modulus were observed for yoghurt samples obtained with the 1% of sh60WPI and sh30WPI addition (Figure 3). During storage at 5°C, the G' values slowly increased for all yoghurt samples, which was probably caused by the stabilization of yoghurt gels by hydro-

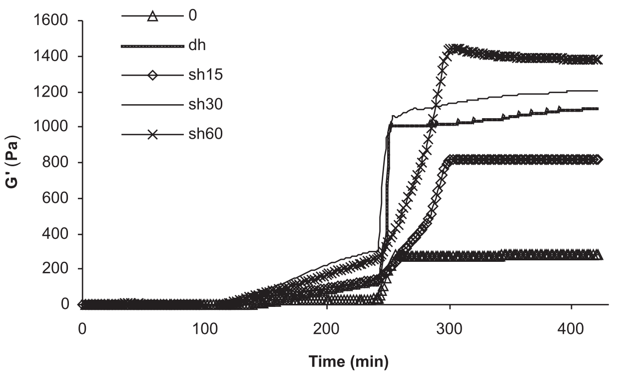

FIGURE 3. Storage modulus (G') of yoghurts during fermentation at 43°C for 240 min and storage at 4°C.

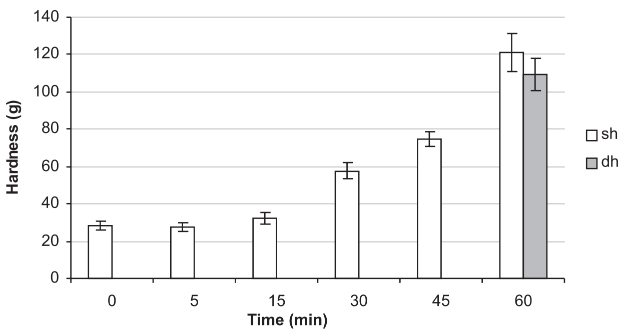

FIGURE 4. Effects of addition of single and double-heated WPI on hardness (g) of set yoghurts.

gen bonds [Gustaw *et al.*, 2006]. The elongation of polymerisation time caused an increase in yoghurts' storage modulus. These observations are in agreement with those reported by Mleko & Gustaw [2000] for whey protein polymer desserts.

The hardness of set yoghurt

The values of set yoghurt hardness are shown in Figure 4. In general, the hardness increased with the elongation of WPI polymerisation time. The hardness of yoghurt with 1% addition of dhWPI was measured at 110 g, but for yoghurts with 1% addition of sh60WPI it was 120 g, respectively. Glibowski *et al.* [2006] reported that gels obtained from double-heated WPI dispersions exhibited lower values of shear stress and strain at fracture than the single-heated dispersions. The comparison of the yoghurts' texture enables observing that hardness of the yoghurts with the dhWPI or sh60WPI addition was four times higher than the one recorded for the control samples. The addition of whey protein aggregates probably caused an increase in density of random whey protein aggregates in yoghurt. These aggregates act as passive fillers and are unlikely to participate in the gel network formation during acidification [Britten & Giroux, 2001]. Puvanenthrian *et al.* [2002] correlated texture of yoghurt with altered casein to whey protein ratios. As the casein to whey protein ratio decreased the gel strength of the yoghurt was observed to increase.

CONCLUSIONS

1. The addition of single- and double-heated WPI caused a significant increase in hardness and storage modulus of set yoghurts in comparison to control yoghurt samples.

2. The elongated time of single WPI denaturation caused an increase in the quantity of whey protein aggregates.

3. The addition of single-heated WPI significantly improved rheological properties of set yoghurt in comparison with double-heated WPI at the same heating time.

REFERENCES

1. AOAC. Official Methods of Analysis, 14th ed., 1984, Arlington, VA.
2. Britten, M., Giroux H.J., Acid-induced gelation of whey protein polymers: effects of pH and calcium concentration during polymerization. Food Hydrocoll., 2001, 15, 609-617.
3. Dalgleish, D.G., Senarante, V., Francois, S., Interactions between α-lactoabumin and β-lactoglobulin in the early stages of heat denaturation. J. Agric. Food Chem., 1997, 45, 3459-3464.
4. Glibowski, P., Mleko, S., Wesolowska-Trojanowska, M., Gelation of single heated *vs*. double heated whey protein isolate. Int. Dairy J., 2006, 16, 1113-1118.
5. Gonzalez-Martinez, C., Becerra, M., Chafer, M., Albors A., Carot, J.M., Chiralt A., Influence of substituting milk powder for whey powder on yoghurt quality. Trends Food Sci. Technol., 2002, 13, 334-340.
6. Gustaw, W., Glibowski, P., Mleko, S., The rheological properties of yoghurt with incorporated whey protein aggregates/polymers. Milchwissenschaft, 2006, 61, 415-419.
7. Ju, Z.Y., Otte, J., Zakora, M., Qvist, K.B., Enzyme–induced gelation of whey proteins: effect of protein denaturation. Int. Dairy J., 1997, 7, 71-78.
8. Ju, Z.Y, Kilara, A., Textural properties of cold-set gels induced from heat-denaturated whey protein isolates. J. Food Sci., 1998, 63, 288-292.
9. Lucey, J.A., Munro, P.A., Singh, H., Effects of heat treatment and whey protein addition on the rheological properties and structure of acid skim milk gels. Int. Dairy J., 1999, 9, 275-279.
10. Mleko, S., Foegeding, E.A., pH induced aggregation and weak gel formation of whey protein polymers. J. Food Sci., 2000, 65, 139-143.
11. Mleko, S., Gustaw, W., Model whey protein polymer dessert. Milchwissenschaft, 2000, 55, 149-151.
12. Puvanenthiran, A., Williams, R.P.W., Augustin, M.A., Structure and visco-elastic properties of set yoghurt with altered casein to whey proteins ratios. Int. Dairy J., 2002, 12, 383-391.
13. Tamime, A.Y., Robinson, R.K., Yoghurt Science and Technology. 1999, Boca Raton, FL: CRC Press, pp. 22-26.

WPŁYW DODATKU AGREGATÓW BIAŁEK SERWATKOWYCH OTRZYMANYCH METODĄ POJEDYNCZEGO I PODWÓJNEGO OGRZEWANIA NA WŁAŚCIWOŚCI REOLOGICZNE JOGURTÓW WYTWARZANYCH METODĄ TERMOSTATOWĄ

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Celem pracy było określenie wpływu dodatku agregatów białek serwatkowych otrzymanych podczas pojedynczego (shWPI) i podwójnego ogrzewania (dhWPI) roztworów izolatu białek serwatkowych na właściwości reologiczne jogurtów wytwarzanych metodą termostatową. Twardość otrzymanych jogurtów badano przy pomocy analizatora tekstury TA-XT2i. Proces fermentacji jogurtów monitorowano podczas ogrzewania przez 4 godz. w temp. 43°C a następnie przechowywania w 4°C przy pomocy reometru oscylacyjnego. Proces powstawania skrzepu kwasowego rozpoczynał się szybciej w przypadku układów z dodatkiem shWPI i dh WPI w porównaniu do jogurtu kontrolnego. Roztwory WPI poddane ogrzewaniu metodą pojedynczą i podwójną analizowano przy wykorzystaniu SE-HPLC. Wraz ze wzrostem czasu ogrzewania roztworów białek serwatkowych zaobserwowano zwiększenie ilości powstałych agregatów białkowych. Twardość jogurtów wzrastała wraz z wydłużeniem czasu agregacji roztworów białek serwatkowych, zastosowanych w produkcji jogurtów.