DOI: 10.7251/QOL1201005S UDC: 637.146.3.05

Original scientific paper

Production of Low Fat Yoghurt Enriched With Different Functional Ingredients

Milka Stijepić[1], Dragica Đurđević-Milošević[2], JovanaGlušac[1]
[1]Higher Medical School, Prijedor, Bosnia and Herzegovina
[2]Anahem d.o.o., Belgrade, Serbia

Abstract: Due to a growing demand for functional fermented dairy foods with improved nutritional qualities, the food processing industry has prompted to cut down on ingredients such as fat, sugar and additives, thereby necessitating some important changes in sensory qualities that influence consumer acceptance of fermented dairy products. Addition of functional ingredients such as whey protein concentrate (WPC) and honey may improve overall quality of yoghurt. It is well known ability of WPC to support formation of whey protein aggregates which highly improve physical properties of yoghurt. Honey may be an ideal sweetener for yoghurt due to its sugar concentration, low pH and a variety of beneficial nutritional properties.

The aim of the present study was to examine the effect of WPC (1%), as well as combination of WPC and honey (H: 2% and 4%) on the physical and chemical properties of low fat set-style yoghurt during 21 days of storage at 5°C. Yogurt was prepared from milk (1.5% fat), treated on 95ºC for 10 min and yoghurt culture VIVOLAC DriSet Yogurt 442: 10% *Lactobacillus delbrueckii* subsp. *bulgaricus* and 90% *Streptococcus thermophilus* (Vivolac Culture Corporation, Indiana, USA), applying standard manufacturing procedure.

It was concluded that the addition of honey in combination with WPC improved quality of produced yoghurt. On the other side, as honey presents a higher nutrition value ingredient, the addition of different percent of honey in combination with WPC could present a novel formulation for functional fermented dairy food.

Key words: honey, WPC, physical and chemical properties, yoghurt culture, yoghurt

Introduction

The term functional foods was firstly introduced in Japan in the mid-1980s and refers to processed foods containing ingredients that aid specific bodily functions in addition to being nutritious (Hasler, 1998). The global functional foods market continues to be a dynamic and growing segment of the food industry. In recent years, due to numerous healthy effects, different kind of dairy products, but particularly yoghurt, increased their popularity among consumers all over the world. *Previous studies* have *shown that* yoghurt is one of the best sources of calcium, an essential nutrient which can prevent osteoporosis and possible colon cancer (Özer & Kirmaci, 2010).

In the production of yoghurt, heat treatment of milk is one of the most important processing parameters that influences consumer acceptance of final products. It was shown that the heat treatment of milk prior to fermentation increases yoghurt firmness and generally influences rheological properties of yoghurt (Harwalkar & Kalab, 1980) (Lucey, et al., 1999). Heating of milk above 70°C induces denaturation and aggregation of whey proteins and their association with casein micelles (Lucey et al., 1999). Addition of functional ingredients such as whey protein concentrate (WPC) and honey may improve overall quality of yoghurt. Fortification of milk for yogurt, prior to the heat treatment of milk, with WPC results in higher concentration of denatured ß-lactoglobulin and α-lactalbumin which saturate all of binding capacity of κ-casein to whey proteins, lead to the formation of additional whey protein aggregates (Puvanenthiran et al., 2002) (Aziznia et al., 2008). An increase in concentration of WPC yielded more compact structure with numerous small pores and a dense network of cross-links (Bönish et al., 2007) which increased firmness (Augustin et al., 2003) (Puvanenthiran et al., 2002), decreased syneresis and increased water holding capacity of the yoghurt (Sahin & Ozdemir, 2004). Since the yoghurt milk is heated at relatively high temperatures (e.g., 90–95°C for 10 min), the whey protein present in WPC is flocculated, and the water-holding capacity of the curd is reduced (Parnell-Clunies et al., 1986). Due to that, it was demonstrated that the heat treatment at 90°C for 10 min is the optimum heat treatment norm to obtain yoghurt with a good textural quality (Yildiz, 2010). Obviously, WPC as an ingredient improves nutritional values and biological effects of **yoghurt** on health (Antunes et al., 2005) (Özer & Kirmaci, 2010) but also may affect consumer's acceptability and preference (Fox, 2001) (Warner et al., 2001). In sensory evaluations, specifically flavour perception, WPC has limited applications in food: 2-3% (Gonzalez-Martinez, 2002), cause may affect undesirable flavour (Quach et al., 1999). Combination with other ingredients such as honey may improve flavour profile. From this point of view, honey may be an ideal sweetener for yoghurt due to its sugar concentration, low pH and a variety of beneficial nutritional properties. Scientific evidence has demonstrated a number of health benefits of honey, as antimicrobial, antiviral, antiparasitory, anti-inflammatory, antioxidant, antimutagenic and antitumor effects (Bogdanov et al., 2008). Other important effects of honey on human digestion have been linked to improvement of calcium and magnesium absorption, prevention of colon cancer and lowering cholesterol (Bogdanov et al., 2008) (Molan, 1997).

Furthermore, several studies have been reported that honey had beneficial effects on the fermented milks; improves LAB viability and longer shelf life (Riazi & Ziar, 2008); improves viability of bifidobacteria in probiotic fermented milks (Chick et el., 2006) (Varga, 2006) and improves the quality of the finished product (Varga, 2006).

The aim of the present study was to examine the effect of WPC, as well as combination of WPC and honey, on the physical and chemical properties of low fat set-style yoghurt during refrigerated storage. On the other side, as honey presents a higher nutrition value ingredient, the addition of different percent of honey in combination with WPC could present a novel formulation for functional fermented dairy food.

Materials and methods

Materials

Homogenised raw milk (1.5% fat, 3.3% proteins, 4.7% lactose), obtained from „MILKO", d.d. Prijedor (Prijedor, Bosnia and Herzegovina), was used for the production of yoghurt samples. The initial pH of the yoghurt milk was 6.58 (±0.01). Physical, chemical and microbiological characteristics of milk samples were entirely in accordance with the pertinent standards.

Yoghurt starter culture VIVOLAC DriSet Yogurt 442: 10% *Lactobacillus delbrueckii* subsp. *bulgaricus* and 90% *Streptococcus thermophilus* (Vivolac Culture Corporation, Indiana, USA) was applied to achieve a concentration of 0.0025 in manufacturing yoghurt samples.

Whey protein concentrate (Textrion™ PROGEL 800 – DMW International BV – Veghel - The Netherlands) contained 80% proteins, 5% ash, 5% fat, and 7% lactose.

Acacia honey (BK Kompani, Bosnia and Herzegovina) declared as follows: 16.9% water, 0.08% ash, 82.6% total sugar, 71.1% reducing sugars), was used. Physical, chemical and microbiological characteristics of honey samples were entirely in accordance with the pertinent standards.

Yoghurt manufacturing

Raw and homogenized skim milk (1.5% fat) with a supplement of WPC (1%) was heated at 95ºC for 10 min. After being heated, milk was cooled to 55ºC and acacia honey was added to the samples with the WPC, so that the two concentrations: 2% and 4% were achieved. As the control samples those without any addition were considered. The milk was cooled to the optimal temperature (41°C), inoculated with the chosen yoghurt starter, poured into 200-ml sterile cups and 50 ml capacity centrifuge tubes (SIGMA, Germany) and incubated at the same temperature until pH 4.5 to 4.6 was reached. Fermentations were stopped by rapid cooling to 20°C and the samples of fermented milk were placed in a cold storage at 5°C ±1. Each trial was repeated three times.

Codes of different yoghurt samples, according to the plan of experiments were: control (control yoghurt without any addition), WPC1 (yoghurt with 1%WPC), WPC1H2 (yoghurt with 1%WPC and 2% honey) and WPC1H4 (yoghurt with 1%WPC and 4% honey).

Physical and chemical analyses

After manufacturing, low fat yoghurt samples were analyzed to determine their physical and chemical characteristics. Physical characteristics were assessed through pH, water holding capacity (WHC) and viscosity, while chemical properties were assessed by measuring lactic acid. pH was measured using pH 510/mV Meter (Eutech Instruments, England) during fermentation and during 21 days of storage. To determine WHC set yoghurt samples were analyzed by the centrifugation method (SIGMA 2-6 Laboratory Centrifuges, Sigma Laborzentrifugen GmbH, Germany). Water-holding capacity (WHC) was determined according to the procedure introduced by Guzman-Gonzalez *et* al. (1999) (Guzman-Gonzalez et al., 1999). Viscosity was measured using a Brookfield DV-E viscometer (Brookfield Engineering Laboratories, Stoughton, MA, USA). The viscometer was operated at 20 rpm (spindle #4). Each result was recorded in mPa·s after a 30 s rotation, during 3 min. Lactic acid is calculated on the basic titratable acidity (Sabadoš, 1996) during 21 days of storage. Analyses of the produced samples were carried out on the 1, 7, 14 and 21 days of storage at 5ºC. The average value of 3 measurements was taken for further analysis.

Statistical analyses

The data was analyzed by using Tukey's test (SigmaPlot 11.0, Sysstat Software, Inc. USA) and Excel 2007. Values of different tests were expressed as the mean ± standard deviation (x ± SD).

Results and discussion

Fermentation

During the first 60 min of fermentation, the pH value of milks supplemented with WPC or WPC and honey was same as control (Fig.1). After 100 min of fermentation of samples supplemented with combination of WPC and honey or WPC alone pH value decreased faster than in control samples. As it is evident from the Fig.1, the fermentation time became shorter for the yoghurt samples with the addition of WPC as well as for the samples with added combination of WPC and honey. The fermentation lasted about 420 min in the control yoghurt, about 300 min in samples with WPC and combination of WPC and 2% honey, and the shortest fermentation time had the samples with combination of WPC and 4% honey, about 4 h (240 min).

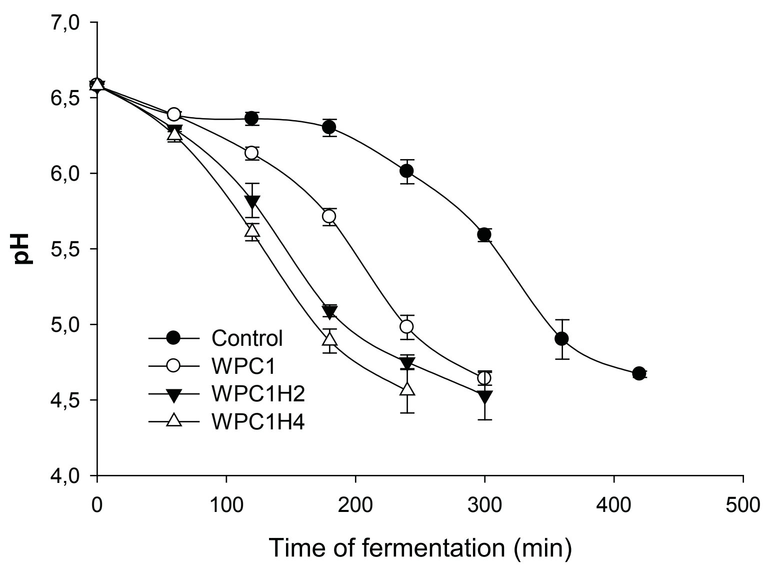

Figure 1. Changes of the pH during milk fermentation with WPC and combination of WPC and honey

The obtained results for samples enriched with WPC are in agreement with the results of some authors (Milanović et al., 2009) (Lucey et al., 1999). They found that the addition of WPC to milk and subsequent heat treatment resulted in a decrease of the gelation time. On the other side, some results of investigation of influence of addition of sunflower honey to milk prior to fermentation have shown that honey did not decrease fermentation time of yoghurt (Sert et al., 2011), which is not in agreement with the obtained results.

pH and lactic acid

The pH and lactic acid (%) of all samples had slightly changed during the storage period (Table 1,2). The pH value during 21 days of storage was in range from 4.49 to 4.21 for control, from 4.48 to 4.14 for samples with WPC, for samples with combination of WPC and 2% honey from 4.34 to 4.08 and for sample with combination of WPC and 4% honey from 4.17 to 3.99 (Table 1). Similar results were obtained by Varga (2006) with yoghurt enriched with 1, 3 and 5% acacia honey during the storage time. The pH values were more stable and lower in the yoghurts with WPC and combination of WPC and honey addition than in control yoghurt. Due to buffering capacity of WPC (Puvanenthiran et al., 2002) yoghurt samples had a stable pH value during the storage time.

Table 1: Influence of added whey protein concentrate (WPC) and added combination of WPC and honey (H) on pH value of low fat yoghurt during storage time

Yoghurt samples	Days of storage 1 7 14 21							
	*☒±SD	Cv	☒±SD	Cv	☒±SD	Cv	☒±SD	Cv
Control	4.49±0.07	1.88	4.29±0.01	0.45	4.25±0.03	1.17	4.21±0.02	0.68
WPC1	4.48±0.08	1.40	4.29±0.03	1.07	4.26±0.03	1.00	4.14±0.03	2.07
WPC1H2	4.34±0.06	2.02	4.18±0.09	1.96	4.14±0.10	2.59	4.08±0.04	1.46
WPC1H4	4.17±0.05	0.89	4.10±0.05	1.45	4.04±0.08	2.10	3.99±0.07	1.90

**Means of lactic acid (±SD), Cv-coefficient of variability*

The data obtained for lactic acid (Table 2) was for control yoghurt from 0.66 to 0.78%, for samples enriched with WPC from 0.69 to 0.80%, for samples with combination of WPC and 2% honey form 0.72 to 0.84% and for samples with combination of WPC and 4% honey from 0.76 to 0.88% (Table 2). Yoghurt enriched with combination of WPC and 4% honey had the highest value of lactic acid during the storage time.

Table 2: Influence of added whey protein concentrate (WPC) and added combination of WPC and honey (H) on lactic acid (%) of low fat yoghurt during storage time

Yoghurt samples	Days of storage 1 7 14 21							
	*☒±SD	Cv	☒±SD	Cv	☒±SD	Cv	☒±SD	Cv
Control	0.66±0.01	1.58	0.70±0.03	4.73	0.73±0.04	4.07	0.78±0.03	3.58
WPC1	0.69±0.01	1.45	0.77±0.02	2.16	0.77±0.03	3.29	0.80±0.02	2.07
WPC1H2	0.72±0.01	1.43	0.79±0.01	1.38	0.82±0.01	1.88	0.84±0.03	3.86
WPC1H4	0.76±0.003	3.54	0.82±0.03	3.62	0.86±0.04	3.98	0.88±0.03	3.66

**Means of lactic acid (±SD), Cv-coefficient of variability*

Viscosity and water holding capacity

Viscosity of yoghurt samples are presented in Figure 2. Produced yoghurt samples had very high viscosity. Control sample had the lowest viscosity (170.4 mPas at the first day and 152.3 mPas at 21 days of storage), while the highest viscosity had sample with combination of WPC and 4% honey (280.5 mPas at the first day and 217.2 mPas at 21 days of storage) apart from the last day of storage when the highest viscosity had a samples with lower addition of honey (2%) in combination with WPC (248.3 mPas).

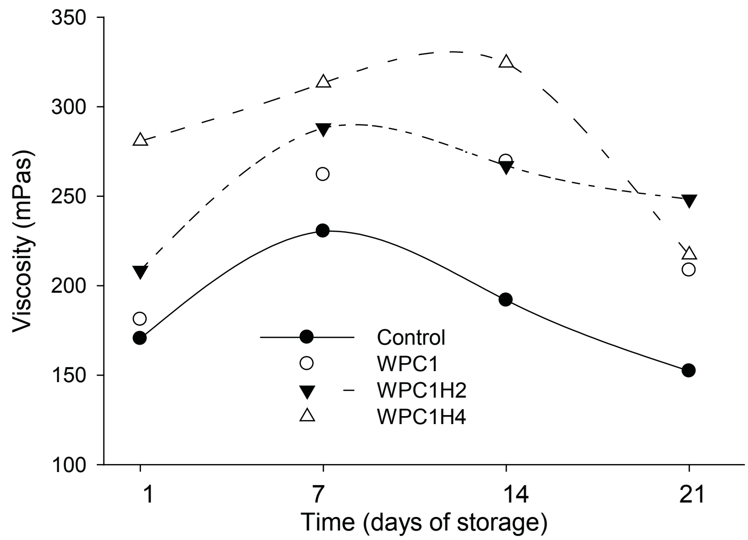

Figure 2. Viscosity of low fat yoghurt with WPC and combination of WPC and honey during storage time

Addition of 2% acacia honey and 1% WPC increased viscosity of yoghurt compared to the control samples, until an increase of honey proportion resulted in a decrease of viscosity. However, this viscosity changes induced by addition of different quantity of honey appeared to have no significant differences ($P>0.05$) compared to the control yoghurt. Viscosity of all samples slightly increased with storage time due to the presence of WPC and thanks to its well known characteristics (Özer et al., 2010) (Augustin et al., 2003). On the other side, due to the presence of honey and water-binding capacity of fructose, viscosity development is a more rapid (Staley, 1987). Furthermore, due to the content of non-digestible dietary fibre (oligosaccharides), honey could probably have similar effect on physicochemical properties of low fat yoghurt as fructooligosaccharides. In previous studies, it has been shown that some fructooligosaccharides could be used in yoghurt production as fat replacer or as stabilizers (Franck, 2002) due to their basic func-

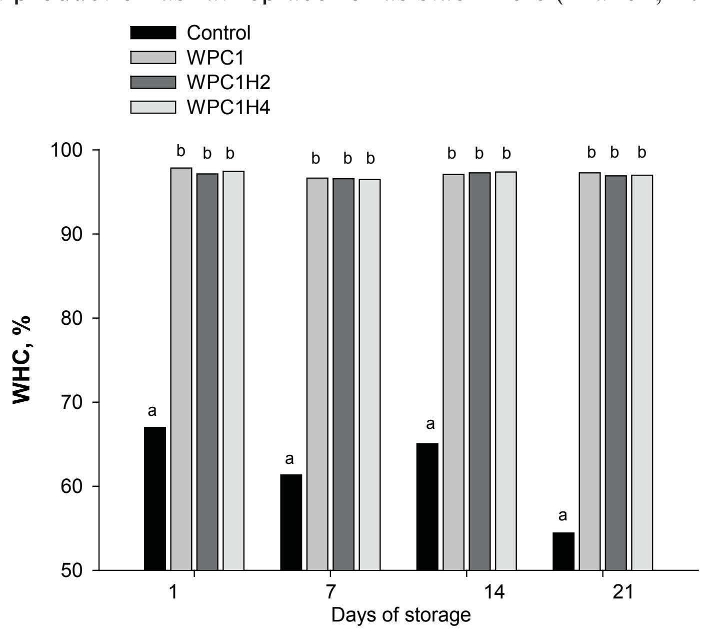

Figure 3. Water-holding capacity of low fat yoghurt enriched with WPC and combination of WPC and honey during storage time [a,b]Means followed by the same are not significantly different (p<0.05) according to Tukey's test

tions: the binding of water and promotion of increase in viscosity (Tamime & Robinson, 1999). This could explain increase in the viscosity of yoghurt samples containing 2% honey, at the end of the storage time. However, higher content of honey (4%) in combination with 1% WPC yielded to lower porosity of protein matrix, weaker protein interactions and strength of protein network which resulted in lower viscosity.

Changes in WHC of yoghurt samples are presented in Figure 3. The lowest WHC had control yoghurt samples and have decreased during storage time from 67.0% (at the first day) to 54.43% (at the end of the storage time). Significantly higher WHC, with about 30% percent higher value of WHC, had the samples with WPC and combination of WPC and WPC and honey whose WHC slightly decreased during the storage time. WPC had significantly increased water-holding capacity even at 0.3 or 0.5% addition and it was about 75% (Milanović et al., 2009). Stable values of WHC of yoghurt samples enriched with WPC during the storage time could be explained by the fact that WHC can be increased by adding stabilisers that interact with the casein network, which in this case is WPC, but also honey. In most honeys, fructose predominates (White, 1980), and fructose has a high water-binding capacity so it may react with many starches. Addition of honey in combination with WPC could lead to a higher water-holding capacity which is connected, also, with a decrease in syneresis due to its rearrangements in protein network structure during the storage time.

Conclusion

Obtained results revealed that WPC addition had positive effects on the physical-chemical properties of produced yoghurt. Water-holding capacity, pH and lactic acid content and viscosity of the products remained almost unchanged after addition of honey. The knowledge obtained from this study could be applied for the development of novel formulation for functional fermented dairy food: WPC and honey enriched yoghurts.

References

Antunes, A.E.C., Cazetto, T.F., & Bolini, H.M.A. (2005). Viability of probiotic microorganisms during storage, postacidification and sensory analysis of fat-free yoghurts with added whey protein concentrate. International Journal of Dairy Technology, 58, 169-173.

Augustin, M.A., Cheng, L.J., Glagovskaia, O., Clarke, P.T., & Lawrence, A. (2003). Use of blends of skim milk and sweet whey protein concentrates in reconstituted yogurt. Australian Journal of Dairy Technology, 58, 30-35.

Aziznia, S., Khosrowshahi, A., Madadlou, A., & **Rahimi, J.** (2008). Whey Protein Concentrate and Gum Tragacanth as Fat Replacers in Nonfat Yogurt: Chemical, Physical, and Microstructural Properties. J. Dairy Sci., 91, 2545-2552.

Bogdanov, S., Jurendic, T., Sieber, R. & Gallmann, P. (2008). Honey for Nutrition and Health. American Journal of the College of Nutrition 27 677-689.

Bönish, P.M., Huss, M.P., Weitl, K., & Kulozik, U. (2007). Transglutaminase cross-linking of milk proteins and impact on yoghurt gel properties. International Dairy Journal, 17, 1360–1371.

Chick, H., Shin, H.S., & Ustunol, Z. (2006). Growth and Acid Production by Lactic Acid Bacteria and Bifidobacteria Grown in Skim Milk Containing Honey. Journal of Food Science, 66, 478–481.

Fox, P.F. (2001). Milk **protein**s as food ingredients. International Journal of Dairy Technology, 54, 41–55.

Franck, A. (2002). Technological functionality of inulin and oligofructose. British Journal of Nutrition, 87, 2, S287–S291.

Gonzalez-Martinez. C., Becerra, M, Chafer, M., Albors. A., Carot, J.M. & Chiralt, A. (2002). Influence of substituting milk powder for whey powder on yoghurt quality, Trend Food Science Technology, 13, 334–340.

Guzman-Gonzalez, M., Morais, F., Ramons, M., Amigo, L. (1999). Influence of skimmed milk concentrate replacement by dry dairy products in a low fat set-type yoghurt model system. I: Use of whey protein concentrates, milk protein concentrates and skimmed milk powder, J. Sci. Food Agric. 79: 1117-1122.

Harwalkar, V.R., Kalab, M. (1980). Milk gel structure. XI. Electron microscopy of glucono-δ-lactone-induced skim milk gels. J. Texture Stud., 11:35-49.

Hasler, C.M. (1998). A new look at an ancient concept. Chemistry and Industry 3, 84-89

Lucey, J.A., Munro, P.A., & Singh, H. (1999). Effects of heat treatment and whey protein addition on the rheological properties and structure of acid skim milk gels. International Dairy Journal, 9, 275–279

Milanović, S., Iličić, M., Đurić, M., & Carić, M. (2009). Effect of transglutaminase and whey protein concentrate on textural characteristics of low fat probiotic yoghurt. Milchwissenschaft - Milk Science International, 64, 388-392.

Molan, P.C. (1997). Honey as an antimicrobial agent – In Bee Products: Properties, Applications and Apitherapy, (A. Mizrahi and Y. Lensky, ed.) pp. 27-37, Plenum Press, New York, NY.

Özer, B.H., Kirmaci, H.A. (2010). Functional milks and dairy beverages. International Journal of Dairy Technology, 63(1), 1-15.

Parnell-Clunies, E.M., Kakuda, Y., Mullen, K., Arnott, D.R., & deMan, J.M.. (1986). Physical properties of yogurt: A comparison of vat versus continuous heating systems of milk. J. Dairy Sci. 69:2593-2603.

Puvanenthiran, A., Williams, R.P.W., & Augustin, M.A. (2002). Structure and visco-elastic properties of set yoghurt with altered casein to whey protein ratios. International Dairy Journal, 12, 383–391.

Quach, M.L., Chen, X.D., & Stevenson, R.J. (1999). Headspace sampling of whey protein concentrate solutions using solid-phase microextraction. Food Research International, 31, 371-379.

Riazi, A., & Ziar, H. (2008). Growth and viability of yogurt starter organisms in honey-sweetened skimmed milk. African Journal of Biotechnology, 7, 2055-2063.

Sabadoš, D. (1996). Kontrola i ocjenjivanje kakvoće mlijeka i mliječnih proizvoda, 2. dopunjeno izdanje, Hrvatsko mljekarsko društvo RH, Zagreb.

Sahin, H., & Ozdemir., F. (2004). Effect of some hydrocolloids on the rheological properties of different formulated ketchups. Food Hydrocolloids, 18, 1015–1022.

Sert, D., Akin, N., & Dertli, E. (2011). Effects of sunflower honey on the physicochemical, microbiological and sensory characteristics in set type yoghurt during refrigerated storage. International Journal of Dairy Technology, 64 (1) 99-107

Staley Manufacturing Co (1987). Crystalline fructose: a breakthrough in corn sweetener process technology. Food Technology, 41, 66–67 & 72.

Tamime, A.Y. & Robinson, R.K. (1999). Yogurt: Science and Technology. 2nd ed. CRC Press, New York, NY.

Varga, L. (2006). Effect of acacia (Robinia pseudo-acacia L.) honey on the characteristic microflora of yogurt during refrigerated storage. International Journal of Food Microbiology, 108, 272-275.

Warner, E.A., Kanekanian, A.D., & Andrews, A.T. (2001). Bioactivity of milk **protein**s: 1. Anticariogenicity of **whey protein**s. International Journal of Dairy Technology, 54, 151–153.

White, J.W. (1980). Detection of honey adulteration by carbohydrate analysis. Journal – Association of Official Analytical Chemists, 63, 11–18.

Yildiz, F. (2010). Development and manufacture of yogurt and other functional dairy products , CRC Press Taylor & Francis Group.

Recived: 25.01.2012.
Accepted: 20.06.2012.