Development of formulation and technology of fermented dairy beverage for musculoskeletal disease prevention

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Abstract. According to the data of World Health Organization, 20–33% of people across the world suffer from painful musculoskeletal conditions, which lead to restricted mobility, dexterity and functional mobility. The aim of the research was to develop formulation and technology of yogurt for prevention of musculoskeletal disease. The results of sensory characteristics, physico-chemical parameters, rheological characteristics, fatty acid composition have shown that it is possible to create the new product with curcumin, grape seed oil, hyaluronic acid and chondroitin sulfate, which are recommended to use for improving various symptoms of musculoskeletal disease. However, the chosen components increase the manufacturing process. It was observed that fermentation time increase was caused by addition of curcumin, which inhibits the lactic acid bacteria growth within 2.5–3 h. The combination Tween 80 and lecithin allows to obtain stable product during the storage period.

Key words: musculoskeletal disease prevention, curcumin, grape seed oil, emulsion.

INTRODUCTION

Musculoskeletal health was included as a noncommunicable disease target since 2016 in the Action plan for the prevention and control of noncommunicable diseases in the WHO (2016) European Region. The Global Burden of Disease study reported that the 20–33% of people around the world suffers from a painful musculoskeletal condition (GBD, 2017).

Musculoskeletal conditions can be caused or made worse by many factors. They can be divided into three categories. The first is professional and work-related factors.

Studies have shown that long-term repetitive operations, incorrect postures, long-term repeated lifting, pushing, pulling, and transporting heavy objects increase the incidence of musculoskeletal injuries (Bernard, 1997; Podniece & Taylor, 2008; Okunribido & Wynn, 2010; Ge et al., 2018).

The second is the individual factor. Factors such as age, gender, genetics, smoking, alcohol, poor nutrition, low phisical activity, hormones, inadequate dietary intake of calcium and vitamin D, overweight and obesity, ethnicity (Karponis, 2004; Guh et al., 2009; Arthritis research UK, 2017; Ge et al., 2018). The third is social and psychological factors, such as job satisfaction, high workload, work monotony, job control, and social support, depression (Arthritis research UK, 2017; Ge et al., 2018).
Musculoskeletal conditions are one of the leading causes of morbidity and disability, giving rise to huge economic burdens to the country and enterprises. The pain and disability caused by musculoskeletal diseases result in a substantial loss in quality of life. Musculoskeletal conditions are a leading cause of work limitations and working days lost (Wooff & Pfeifer, 2003; Buckle, 2005; Miller et al., 2005, Arthritis research UK, 2017; Briggs et al., 2018; Ge et al., 2018).

In this study yogurt was chosen as a vehicle for the oral administration of these natural components. Yoghurt is one of the highly-consumed dairy foods in the world and it also demonstrates unique health benefits. It contains Lactobacillus subsp. bulgaricus, which is one of the first probiotic strains ever studied. It also helps reduce the risk of caries, calcium and vitamin D deficiency, reduce body mass index, blood pressure, sugar levels (Burgain, 2012; Kim, 2013; Wu et al., 2013). For this investigation yogurt and grape seed oil were also selected based on the recommendation to consume curcumin with milk and with any edible oil (Gouda & Bhandary, 2018).

Curcumin (diferuloylmethane) is a yellow pigment isolated from Curcuma longa L. (turmeric) (Gutierrez et al., 2015). Curcumin is stable during thermal treatment. Results of numerous research have showed that curcumin can reduce severity of pain in adults suffer from arthritis in comparison with placebo or analgesic medications and improve, functional outcomes (Madhu et al., 2012; Lakhan et al., 2015; Daily et al., 2016; Ross; 2016). Besides, curcumin shows significant antioxidant, antancer, anti-inflammatory, antimutagenic, antimicrobial, gastroprotective properties and play an in the prevention and treatment of diabetes, and its associated disorders (Zhang et al., 2013; Jovičić et al., 2017). Research suggests that curcumin can help in the management of oxidative and inflammatory conditions, metabolic syndrome, arthritis, anxiety, and hyperlipidemia. It helps to protect liver, brain, age-related diseases and cancer (Ferrari, 2013; Pulido-Moran et al., 2016). Dietary intake of isolated curcumin could be more effective compared to whole turmeric powder. It is recommended to consume curcumin with milk, pepper, honey or with any edible oil (Gouda & Bhandary, 2018).

Hyaluronic acid is a glycosaminoglycan, which is a substance that attaches to collagen and elastin to form cartilage and also helps to increase supplies of joint-lubricating synovial fluid, thus protecting the articular cartilage. Due to its viscoelasticity, it absorbs mechanical impacts and avoids friction between the bone-ends (Fallacara et al., 2018). The functions of hyaluronic acid include preventing cartilage denaturation, protecting the outer layer of cartilage, blocking synovial inflammation, increasing chondrocyte density, promoting synovium metabolism, normalizing synovial fluid, and treating sharp pain (Oe et al., 2016).

Therefore, without adequate amounts of hyaluronic acid, disorders such as rheumatoid arthritis and osteoarthritis occur, the joints will become brittle and deteriorate (Manasa et al., 2012; Tamer, 2013; Fallacara et al., 2018). Additionally, hyaluronic acid was found to be beneficial also for the treatment of joint (Fallacara et al., 2018). A 70 kg human contains around 15 g total of hyaluronic acid of which about 2–4 g L⁻¹ in the synovial fluid. Hyaluronic acid is also present in skin, blood vessels, serum, brain, heart valves, and the umbilical cord (Frasher et al., 2003; Volpi et al., 2009; Oe et al., 2016).

Chondroitin sulfate is a glycosaminoglycan formed naturally by the body for the synthesis and maintenance of connective tissue. It is also can be extracted from animal cartilage (cows, pigs, birds, and fish) (Jerosch, 2011; Pelletier et al., 2016).
Due to the negative charge of chondroitin sulfate, it is responsible for the water retention of the cartilage, which is important for pressure resistance. Chondroitin sulfate is a symptomatic slow-acting drug. The first effects become noticeable after 2–3 weeks of regular intake and remain for up to several months (Jerosch, 2011). Chondroitin sulfate supports and protects structure and function of cartilage as well as other connective tissue in numerous ways.

Results of many studies demonstrated that chondroitin sulfate has a beneficial effect on pain and joint space narrowing in patients with knee osteoarthritis, decreased cartilage volume loss, improves spinal function and allows to slow down musculoskeletal disease. Chondroitin sulfate inhibits the enzymes leukocyte elastase and hyaluronidase, which are found in high concentration in the synovial fluid of patients with rheumatic diseases (Oliviero et al., 1991; Pepitone, 1991; Bucci & Poor, 1998; Uebelhart, 1998; Shostak et al., 2002, Mazurov & Belyaeva, 2004; Kahan et al., 2009; Jerosch, 2011; Wildi et al., 2011).

Grape seed oil is recognized as beneficial wine industry by-product (Pardo et al., 2009). Besides pleasant sensory characteristics grape seed oil has a high linoleic acid content, high vitamin E and F contents, polyphenols, flavonoids, carotenoids, minerals like zinc potassium, copper, calcium, phosphorus, magnesium, iron and selenium and low values of cholesterol. Grape seed oil is rich in proanthocyanidins which antioxidant properties are 50 times more effective than Vitamin E and 20 times stronger than Vitamin C (Herting et al., 1963; Nash, 2004; Pardo et al., 2009; Erlich, 2012). Grape seed oil has also high concentration of tannins, oligomeric proanthocyanosides at 1,000 times higher than the other oils and that is the reason why it has high stability and resistant to oxidation reaction (Sotiropoulou et al., 2015).

The highlight health benefits that offers the consumption of grape seed oil including antitumor, antioxidant, anti-inflammatory, cardioprotective, neuroprotective, antimicrobial effects which has been proven by preclinical tests and studies in humans (Shinagawa et al., 2015; Garavaglia et al., 2016).

The aim of this study is to investigate the possibility of using the combination of hyaluronic acid, curcumin and chondroitin sulfate in the manufacture of beneficial product, which would help prevent musculoskeletal disease.

**MATERIALS AND METHODS**

Skimmed milk powder (commercial brand Central Lechera Asturiana, Corporación Alimentaria Peñasanta, S.A., Spain, 34 g protein and 52 g carbohydrates per 100 g) reconstituted with distilled water at a temperature 45 °C and stirred for enough time to assure solubilisation and hydration of solids.

**Control sample preparation**

Reconstituted skimmed milk was heated to 90 ± 2 °C for 5–7 min, and then cooled immediately to 42 ± 1 °C. Milk was inoculated with the starter culture at a rate of 3% (*Lactobacillus delbrueckii subsp. Bulgaricus* and *Streptococcus thermophilus*) and fermented at 42 ± 1 °C until the complete coagulation of yoghurt. The yoghurt samples were stored at about 5 °C at refrigeration until used.
**Treatment samples preparation**

Chondroitin sulfate (0.6% (w/v), Now Foods, USA) and hyaluronic acid (0.05% (w/v), Jarrow Formulas, USA) were dissolved in distilled water at a temperature 50 ± 2 °C with holding time 10–15 min. These solutions were added to the reconstituted skimmed milk and mixed for 15 min (1,050 rpm, RZR 2020, Heidolph Instruments GmbH). The amount of water for skimmed milk reconstitution was reduced by the amount required for chondroitin sulfate and hyaluronic acid dissolution. Curcumin (0.3% (w/v), Thompson, USA) was dissolved in grape seed oil (Fratelli Mantova, Italia) at 50 ± 2 °C with holding time 10 min. For the preparation of oil in water (O/W) emulsions, method of two-step mechanical homogenization was used. Based on preliminary studies (data not shown) grape seed oil 7% (w/w) was added gradually and the most suitable homogenization conditions were 6,500 rpm for 5 min (1 step) and 21,500 for 3 minutes (2 step). The emulsification process was carried out using Ultra-Turrax® (IKA T 25, Germany). The homogenization temperature was kept at 50–55 °C. Based on the results of another part of our study concerning lecithin and Tween 80 concentration selection, in order to improve emulsion final stability, lecithin (Cargill Inc., Germany) and Tween 80 (Polyethylene glycol sorbitan monooleate, Sigma-Aldrich) in the ratio 8:2 (1.2% v/v) were added to the formula. Further step were made according to the same process of control sample manufacture. The amount of starter culture varied from 3% to 5%, with the increment of 1%.

**Methods**

pH values were measured using pH–meter pH–410 with combined glass electrode (Scientific Production Association ‘TECHNOKOM’, Russia).

Titratable acidity was measured according to AOAC method 947.05 (AOAC, 2007).

Centrifugally separated whey was estimated visually and by the amount of separated whey during the centrifugation of 10 g of yoghurt. Each sample was weight into centrifuge tubes, centrifuged and the amount of separated whey was measured every 5 min during 30 min.

The rheological measurements were performed at controlled temperature of 20.0 ± 0.2 °C and 4 ± 2 °C using rotational rheometer Rheotest RN 4.1 (RHEOTEST Medingen GmbH, Germany). Since the structure of yoghurt gel is sensitive to its shear deformation history, samples were carefully loaded to the rheometer. The measurement were taken using spindle (II), sample were subjected to shear rates ranging from 0.11 to 20.43 s⁻¹. Rheological analysis was carried out after 24 hours of yoghurt production. Before measurements, yoghurt samples were gently stirred with a spatula for 30 seconds to ensure homogeneity.

Vitamin E concentration was determined according to M 04-10-2007 using HPLC ‘Lumachrom’. The method based on sample alkaline hydrolysis. As a result, in conjunction with saponification vitamin essential form lipid transformation in alcohol forms occurs; hexane extraction followed by separation and determination of vitamin E using HPLC method with fluorimetric detection.

Fatty acid composition of yoghurt was analysed was determined by gas chromatograph (GC-2010, Shimadzu, Tokyo, Japan) equipped with a flame ionization detector and a capillary column DB-23 (60 m x 0.25 mm x 0.25 µm) (Agilent Technologies, Santa Clara,CA, USA). Injector and detector temperatures were set as 250 °C and 280 °C, respectively.
Emulsion stability of control and treatment samples of yogurt was determined experimentally by the measurement of the creaming index (CI) (Eq. 1) in 24 h after yogurt manufacture and during the storage period every 5 days. An emulsion (35 mL) was placed into a 50 mL centrifugal plastic tube and centrifuged at 2,500 rpm for 15 min at 25 °C. An emulsion (30 mL) was placed into a 50 mL centrifugal plastic tube and centrifuged at 7,500 g for 15 min at 25 °C. The CI values were obtained from the ratio between the total height of cream layer (CC) and the total height of emulsion layer (TE).

$$CI(\%) = \frac{CC}{TE} \cdot 100$$

*Lactobacillus delbrueckii subsp. Bulgaricus* and *Streptococcus thermophilus* were counted according to GOST 33951-2016.

Organoleptic characteristics were assessed using 9-point hedonic scale (Clark et al., 2009) with four positive categories in the upper pole, a centered neutral category and four negative categories in the lower pole (Table 1). Yogurt sample were assessed by a 36-member panel (50% of participants were female from 20 to 70 years old and 50% of participants were male from 19 to 65 years old) selected from students and staff members of our Faculty and were unsalted cracker for palate cleansing between the samples.

<table>
<thead>
<tr>
<th>Score</th>
<th>Categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Like extremely</td>
</tr>
<tr>
<td>8</td>
<td>Like very much</td>
</tr>
<tr>
<td>7</td>
<td>Like moderately</td>
</tr>
<tr>
<td>6</td>
<td>Like slightly</td>
</tr>
<tr>
<td>5</td>
<td>Neither like nor dislike</td>
</tr>
<tr>
<td>4</td>
<td>Dislike slightly</td>
</tr>
<tr>
<td>3</td>
<td>Dislike moderately</td>
</tr>
<tr>
<td>2</td>
<td>Dislike very much</td>
</tr>
<tr>
<td>1</td>
<td>Dislike extremely</td>
</tr>
</tbody>
</table>

**Table 1.** Numerical and verbal representations of the 9-point hedonic scale

Statistical analysis

All measurements were replicated at least 3 times for each sample.

All experiments were performed at least in triplicate. Data were processed by methods of mathematical statistics at theoretical frequency 0.95. Statistical processing of data was carried out using computer programs Microsoft Office Excel 2010 and Mathcad 15.0.

RESULTS AND DISCUSSIONS

Values presented in Figs 1, 2 showed the pH and titratable acidity changes during fermentation process.

During the fermentation GSO, HA slightly affect the changes in titrated acidity compared to the control sample. HS decrease the fermentation by about 30 minutes. The combination of all additives (sample with GSO, HA, CS and curcumin) significantly decrease the process of fermentation.

In the samples with GSO and HA, the mean pH value changes were close to the control sample. In the sample with HS, the pH drops slower. In general, the pH values of the treatment sample with GSO, HA, CS and curcumin decreased significantly slower during fermentation in comparison with other treatment and control samples.
**Figure 1.** Changes in pH during fermentation: ◆ – control sample; ■ – sample with HA; ▲ – sample with CS; ✥ – sample with CS, GSO, HA, curcumin; ✧ – sample with GSO.

**Figure 2.** Changes in titratable acidity during fermentation: ◆ – control sample; ■ – sample with HA; ▲ – sample with CS; ✥ – sample with CS, GSO, HA, curcumin; ✧ – sample with GSO.

Increasing the amount of starter affects the time of fermentation (Figs 3, 4). However, during the first 3 hours, the titratable acidity and pH, regardless of the amount of the starter, change in the same way. In further, the time of fermentation varies depending on the amount of the added starter: an increase in the amount of starter by 1% reduces the time of fermentation by 1 hour. However, the fermentation time is equal at least 6 hours.

This increasing in fermentation time is probably caused by the addition of curcumin and lactic acid bacteria need more time for adaptation (approximately 2.5–3 h) compared to the plain yogurt. In other words, curcumin does not allow bacteria to develop immediately, as occurs in the control sample, they begin to increase population only after adaptation to the environment.
Figure 3. Effect of starter culture amount on pH-values of yogurt during fermentation:
- control sample; ▲ - treatment sample with 3% of starter culture; ■ - treatment sample with 4% of starter culture; ● - treatment sample with 5% of starter culture.

In 5 hours of fermentation (time is needed for the complete fermentation of the control sample with 1% of starter culture) the lowest numbers of *Lactobacillus delbrueckii subsp. bulgaricus* were observed for the treatment sample (1% of starter culture). The numbers of *Lactobacillus bulgaricus* for the control and treatment samples were $5.0 \times 10^8$ CFU mL$^{-1}$ and $5.0 \times 10^6$ CFU mL$^{-1}$, respectively. The numbers of *Streptococcus thermophilus* for the control and treatment samples were $1.1 \times 10^8$ CFU mL$^{-1}$.

The results of water holding capacity of the control and treatment samples are shown in Fig. 5.

The experimental results can verify the water holding capacity and, therefore, the porosity of the yogurt samples as well. The amount of liquid separated from the samples affirmed that the yogurt with addition of CS, HA or GSO are more resistant against whey explosion. The results of centrifugally separated whey are presented in Fig. 5.

Based on the results (Fig. 5), the sample with GSO had the smallest amount of whey expelled indicating that this sample had the highest water holding capacity, while the
control sample had the smallest water holding capacity. Yogurt samples with addition of CS showed a water holding capacity close to that of the control sample. The decrease in the amount of whey expelled in yogurt samples with addition of CS can be due to the negative charge of CS (Jerosch, 2011). Concerning the sample with HA, the less amount of whey can be caused by hydrogen bond formation between water molecules and adjacent carboxyl and N-acetyl groups of HA (Manasa et al., 2012).

![Graph showing water holding capacities of yogurt samples](image)

**Figure 5.** Water holding capacities of yogurt samples: ⋄ – control sample; ■ – sample with HA; ▲ – sample with CS; × – sample with CS, GSO, HA, curcumin; ◊ – sample with GSO.

Yogurt apparent viscosity has been evaluated 4 °C and 20 °C. The data are shown in Fig. 6 and Fig. 7. The apparent viscosity of the control and treatment samples decreased as the shear rate increased due to shear thinning behaviour and implied that the fluid does not have a true viscosity. Since apparent viscosity is affected by different factors such as milk composition, heat treatment of milk and additives, the apparent viscosity of the of treatment sample showed significant lower values than those of control sample. These results could be due to the intermolecular bonds reduction in treatment sample. The results obtained for the treatment sample found the same tendency for changes in the rheological properties of the control sample in both cases understudy, despite the component introduction.

![Graph showing dependence of apparent viscosity on shear rate](image)

**Figure 6.** Dependence of the apparent viscosity of yogurt samples on the shear rate at 4 °C.
Figure 7. Dependence of the apparent viscosity of yogurt samples on the shear rate at 20 °C.

By increasing shear rate from 0.11 to 1.17 s⁻¹, the enriched yogurt showed a greater decrease in apparent viscosity as compared to the control (Figs 6, 7), indicating faster disruption of protein aggregates in the enriched yogurt.

The addition of GSO (Table 2) can achieve fortified yogurt containing omega-3 polyunsaturated fatty acid. The contents of most important fatty acids for human health—C18:1, C18:2 were significantly higher in the treatment yogurt than in skimmed yogurt. The incorporation of GSO also allows to fortify yogurt with vitamin E. As the recommended daily intake of vitamin E (Institute of Medicine. Food and Nutrition Board, 2000) is 15 mg for an adult the developed product (100 g) satisfies the daily intake for vitamin E up to 26.7%.

The results of the short term stability study are shown in Fig. 8. According to data, the emulsions showed different behavior to creaming during the storage time. The higher the creaming index value is, the more destabilized the emulsion is. Regarding the creaming index data, the lowest CI rates were observed at time 1 day for the treatment sample with emulsifiers, where CI was 1.01%. After 25 days, lower CI was also observed for the treatment sample with selected emulsifiers (2.63%). It can be concluded that yogurt samples made with the mixture of Tween 80 and lecithin (2:8) are more stable than those made without emulsifiers.

<table>
<thead>
<tr>
<th>Table 2. Fatty acid composition of the treatment yogurt</th>
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<tr>
<td>Fatty acid profile/Vitamin E</td>
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<tr>
<td>Palmitic</td>
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<tr>
<td>Palmitoleic (16:1)</td>
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<tr>
<td>Stearic (18:0)</td>
</tr>
<tr>
<td>Oleic (18:1)</td>
</tr>
<tr>
<td>Linoleic acid (18:2) (w-6 PUFA)</td>
</tr>
<tr>
<td>Linolenic (18:3)</td>
</tr>
<tr>
<td>Arachidic (20:0)</td>
</tr>
<tr>
<td>Gadoleic (20:1)</td>
</tr>
<tr>
<td>Vitamin E</td>
</tr>
</tbody>
</table>
Figure 8. Creaming index versus days of storage: ■ – treatment sample without emulsifiers; □ – treatment sample with emulsifiers.

Data from the Fig. 9 present the sensory evaluation of yogurt samples. It can be seen, that the mean scores for overall acceptance obtained for treatment sample was higher than for the control sample. In terms of appearance, treatment sample presented slightly lower degree of liking by the panelists, reflecting differences in outcomes by age or sex among judges. The bright yellow color of the treatment sample gained higher scores among people at the age between 19–49. In terms of taste and flavor all yogurt samples were well accepted by the panelists, and the treatment sample was the product of greater acceptance.

Figure 9. Hedonic scale for appearance and overall acceptability of the samples: ■ – control sample; □ – treatment sample with emulsifiers.

Consequently, sensory analysis indicated that chosen sensory attributes and overall acceptability were very satisfactory and graded with the highest score treatment sample.

CONCLUSIONS

The present work demonstrated the feasibility of yogurt with incorporated curcumin, hyaluronic acid, chondroitine sulfate and grape seed oil. The results indicate that it is possible to produce product of good sensory quality for the prevention of musculoskeletal disease prevention. The fortification of yogurt with chosen components
allows to increase the storage period of yogurt in comparison to the plain yogurt, 10 and 21 days, respectively.

The selected components improve the properties of yogurt, however, affect the time of its manufacture. The developed technology and formulation can be an excellent outlet for the use of valuable skimmed milk solids. The developed product is a good source of vitamin E and w-6 PUFA. The combination of emulsifiers could be recommended for creation stable product during the storage.

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