Performance of bovine and ovine liquid whey protein concentrate on functional properties of set yoghurts

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Abstract

The effects of liquid whey protein concentrates (LWPC) on biochemical, physical and sensorial properties of set yoghurt were studied. Bovine and ovine LWPC were used to partially replace skimmed milk powder (SMP) in bovine yoghurt formulations. The properties of modified yoghurts were evaluated during their shelf-life and compared with conventional bovine and ovine yoghurts. The protein content of ovine yoghurt differs significantly (p<0.05) from the bovine ones (with or without LWPC supplementation). Higher values of hardness, adhesiveness and gumminess were observed for conventional yoghurts, although cohesiveness, resilience and springiness did not vary between formulations. During the products shelf-life a decrease in luminosity was observed, but no significant differences in colour occurred among formulations. Low syneresis indexes, ranging from 0.5 to 5.0%, which are typical in the range of yoghurts with high levels of solids, were achieved for the produced yoghurts. The decrease in viscosity led to an increase in syneresis, indicating that the gel structure was more open retaining water not so efficiently. Ovine yoghurts showed lower syneresis and higher viscosity values, while the yoghurts enriched with LWPC showed the opposite pattern. At the sensory level no differences (p<0.001) were found between conventional bovine yoghurt and yoghurts with LWPC. However, in the case of ovine yoghurt (LO) significative differences were identified, and this product was strongly penalized in the preference test. The results revealed that LWPC (independently of the source) can be used in set yoghurt formulations, increasing protein and total solids content, for total or partial replacement of the conventional adjuvant (SMP). The utilization of these products is very attractive due to the low complexity processing conditions needed, lower production costs and more effective whey disposal.

Keywords: bovine and ovine LWPC; set yoghurt; viscosity; syneresis; textural properties

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1. Introduction

Increasing protein content in yoghurts implies the milk fortification either concentration processes (evaporation or ultrafiltration) or by addition of SMP. More recently, whey protein concentrate (WPC) are also used due to their availability and low cost. Despite WPC are largely applied as attractive food ingredient in a wide range of food applications, the direct reincorporation of liquid whey protein concentrates (LWPC) in dairy products, being a less expensive alternative, is seldom referred. The effect of the replacement of SMP by WPC on textural and physicochemical properties of yoghurts has been reported by several authors [1-4], but in some cases their conclusions were contradictory. The reasons pointed for that are the significant variations in the functionality of WPC resultant from the whey processing conditions, specially heating [5-10] and the whey source [6, 11, 12]. In Portugal, bovine and ovine cheese production represents approximately $60 \times 10^3$ and $15 \times 10^3$ ton/year respectively [13], and the last product is normally associated with Protected Geographical Indication Labels. Based on the cheese production the overall volume of whey produced annually is estimated in approximately 560 000 tonnes. In Mediterranean countries as well as in Portugal, small and medium scale dairy industries represent the majority of producers and face simultaneously environmental problems related to their whey disposal, low production yields and difficulties to succeed in the market due to their specialization in just one product. The use of membrane technologies, namely ultrafiltration (UF) and diafiltration (DF) enables the extraction and concentration of whey proteins from whey to its reincorporation in production, solving their environmental problems and add value to existing products.

No information is available about the LWPC functionality in yoghurt. In this work we intended to evaluate the effects of partial substitution of SMP by LWPC of bovine and ovine origin on physicochemical, textural, rheological and sensorial properties of set yoghurts as well as to test the acceptability of ovine yoghurt as an alternative product in Portugal.

2. Material & Methods

2.1. LWPC manufacture

Bovine and ovine cheese whey were supplied respectively by Queijaria Serqueijos SA (Portugal) and Queijaria Flor da Beira SA (Portugal), obtained immediately after production and transported to the pilot plant in 50 L jars. After reception, the whey was filtered, analyzed and processed. The production of LWPC consisted in whey concentration at 24-30ºC in a batch ultrafiltration pilot plant, using an organic membrane DSS™, model 20K 3838-30, 5.5 m² installed area and 20 kDa cutoff. After concentration, the retentate was submitted to a thermal treatment (90ºC/60s) to precipitate denaturated whey proteins. The mixture was then homogenized at 100 bar to achieve a particle diameter lower than 10 μm in order to avoid disturbance of the casein matrix [14, 15]. Before its incorporation in milk batches, for yoghurt production, the LWPC was analyzed and frozen at -15ºC.

2.2. Set yoghurt manufacture

Four yoghurt formulations with 16% total solids were produced (Table 1). Conventional ovine yoghurts (LO) were produced exclusively with ovine skimmed milk. The formulations with bovine milk were normalized in fat content with cream, and protein content using respectively: (i) SMP (conventional bovine yoghurt (LB)); (ii) 7.3% of bovine LWPC + 4.4% of SMP (LB-LWPCb) and (iii) 7.3% of ovine LWPC + 4.8% of SMP (LB-LWPCo).

All the ingredients for each formulation were mixed, homogenized at 100 bar to achieve a particle diameter lower than 10 μm in order to avoid disturbance of the casein matrix [14, 15]. Before its incorporation in milk batches, for yoghurt production, the LWPC was analyzed and frozen at -15ºC.

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All the ingredients for each formulation were mixed, homogenized at 200 bar and pasteurized at 92°C/30 min. Before filling and packaging, the mixture was stirred, during 20 min, at 43°C and inoculated with a mixed culture of Streptococcus thermophilus and Lactobacillus bulgaricus (Ezal YO-MIX 601).
The fermentation step was performed in 50 mL polystyrene cups at constant temperature of 43±1 °C until the yoghurt pH reached the value of 4.6±1. The yoghurts were then stored at 4±2°C. After one day of cool storage the biochemical composition and functional properties of yoghurt samples were evaluated while the remaining samples were evaluated at the 14th and 21st day.

### Table 1. Yoghurt formulations

<table>
<thead>
<tr>
<th>Composition</th>
<th>LO</th>
<th>LB</th>
<th>LB-LWPCb</th>
<th>LB-LWPCo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovine skimmed milk</td>
<td>100.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bovine milk</td>
<td>-</td>
<td>93.8</td>
<td>88.0</td>
<td>86.7</td>
</tr>
<tr>
<td>Skimmed milk powder (SMP)</td>
<td>-</td>
<td>5.0</td>
<td>4.4</td>
<td>4.8</td>
</tr>
<tr>
<td>Cream</td>
<td>-</td>
<td>1.2</td>
<td>0.3</td>
<td>1.2</td>
</tr>
<tr>
<td>LWPCb</td>
<td>-</td>
<td>-</td>
<td>7.3</td>
<td>-</td>
</tr>
<tr>
<td>LWPCo</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.3</td>
</tr>
</tbody>
</table>

### 2.3. Chemical and functional analyses of LWPC and yoghurts

The biochemical composition (pH, titrable acidity (TA), total solids, ash, fat and protein) of whey, LWPC, milk, skimmed milk powder, cream and yoghurt was evaluated using the Portuguese Standards Methods [16] and the Official Analytical Methods [17]. Each product sample was collected using the Portuguese specific standard procedure for dairy products [18]. For set yoghurt analyses, three samples at random were selected.

Yoghurt colour was determined with a colorimeter Minolta Chroma Meter, model CR-200B, using the L*a*b* CIE LAB system. Syneresis index followed the method described by Gauche (2007). The evaluation of yoghurt viscosity was performed during 10 min (2 min intervals), in a rotational Brookfield Viscometer, model DV II, with a concentric cylinder RV (spindle 3) at a constant angular velocity (5 rpm). A Stable Micro Systems Texture Analyzer, model TA.XT Express Enhanced, was used to perform textural analysis and the results were calculated by the Specific Expression PC Software. For refrigerated yoghurt samples a TPA was run with a penetration distance of 20 mm at 5 mm/s test speed, using an acrylic cylindrical probe with a diameter of 12.7 mm and 35 mm height. For sensorial analysis, triangular tests and preference tests were performed by an untrained panel in order to detect differences between products with conventional formulations and LWPC incorporation. The triangular tests were based in Binomial distribution with a confidence level at p<0.001 [20].

Statistical analysis of the data was carried out using ANOVA package included in Statistica 8 software. Means were compared using the Tukey HSD test. Differences were considered significant at p<0.05.

### 3. Results & Discussion

#### 3.1. Characterization of bovine and ovine LWPC

Table 2 shows the chemical composition of bovine and ovine whey and LWPC after ultrafiltration. Ovine whey was significantly (p<0.05) richer than the bovine whey in all components. The protein and mineral contents in ovine whey (18.6% (dry weigh) and 13% against 12.8% e 7% in bovine whey) indicate that ovine products can be more attractive to achieve higher yields. Comparing both LWPC it was observed that the ovine product presented lower amounts of all the components, except in the case of minerals. The reason for that was the volume concentration factor applied in the UF step that was lower in this case (VCF=13) against (VCF=20) used in the case of bovine LWPC. However, for instance the
protein content still represents higher amounts (36.0% (dry weigh)) than in bovine LWPC (31.3%). Titrable acidity was also higher in the case of ovine whey. The main reason for this could be the type of compounds formed during enzymatic hydrolysis of casein by cardosin (*Cynara cardunculus*), normally used in the manufacture of Portuguese ovine cheeses, against rennet extract (> 96% of quimosin) used for bovine cheese production. The TA reduction in ovine LWPC may be due to higher buffer capacity of ovine proteins.

Table 2. Gross chemical composition of bovine and ovine whey and liquid whey protein concentrate (LWPC): total solids, fat, protein, ash and titrable acidity (TA)

<table>
<thead>
<tr>
<th>(%)</th>
<th>Whey</th>
<th>LWPC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bovine</td>
<td>Ovine</td>
</tr>
<tr>
<td>Total solids</td>
<td>6.92±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.60±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat</td>
<td>0.78±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.45±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein</td>
<td>0.89±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.41±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash</td>
<td>0.50±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.01±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TA (% lactic acid)</td>
<td>0.11±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.13±0.017&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> means of two batches
<sup>a, b</sup> means within the same rows for the same product without the same superscript are significantly different (p<0.05)

3.2. Yoghurt composition and physicochemical properties during storage

The biochemical composition of the four different types of yoghurts prepared according to the formulations presented in Table 1 is shown in Table 3. Ovine yoghurts (LO) only differed from conventional bovine yoghurts (LB) in protein content. In this case, the protein amount (6.06%) was significantly higher than that achieved in bovine yoghurts, being although in the same order of magnitude of similar products [21]. The value of TA achieved in the yoghurts with ovine LWPC incorporation (LB-LWPCo), was significantly lower (p<0.05) from that of the other yoghurt formulations. During storage TA increased for all products, but in a more pronounced way in conventional ones, indicating the higher buffer capacity of LWPC minimizing the yoghurts acidification during storage. This behavior was also reported by Amatayakul et al. (2006) that higher amounts of solids (such as in conventional yoghurts) available during fermentation could promote the increase of microbiological activity and consequently higher production of lactic acid.

Comparing LO and LB yoghurts with the ones produced with LWPC incorporation, no significative differences in color were found. However, during storage the L* value decreased for all the formulations (Figure 1). These results are in accordance with the ones reported by Cais-Sokolinska and Pikul (2006) and Gomes (2010) that concluded that during storage the luminosity of yoghurts tends to decrease.

The textural analysis did not differ over the products shelf life in each type of formulation, neither between formulations for cohesiveness, springiness and resilience. Hardness, adhesiveness and gumminess were significantly higher (p<0.05) for the ovine yoghurts (LO), followed by the bovine yoghurts (LB) and finally by the products with incorporation of LWPC. These results were also observed by de Wit et al. (1986) that confirmed a decrease in hardness by the utilization of WPC with a high protein denaturation level. Other authors concluded that gels produced by the incorporation of previously denatured WPC to milk caseins resulted in less homogeneous and of increased open structure gels then the ones produced by denaturation of whey proteins in the presence of caseins [25]. They suggested that the whey protein aggregates produced during the pre-denaturation step have significative higher dimensions and can not coat properly the caseins, penalizing the gel formation. The LWPC origin (bovine or ovine) did not influence de textural parameters.
Table 3. Gross chemical composition of yoghurts: total solids, ash, fat, protein, titrable acidity (TA) and pH

<table>
<thead>
<tr>
<th>(%)</th>
<th>Yoghurt1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LO</td>
</tr>
<tr>
<td>Total solids</td>
<td>15.97±0.06 b</td>
</tr>
<tr>
<td>Ash</td>
<td>1.04±0.06 ab</td>
</tr>
<tr>
<td>Fat</td>
<td>3.40±0.00 a</td>
</tr>
<tr>
<td>Protein</td>
<td>6.06±0.24 b</td>
</tr>
<tr>
<td>TA (% lactic acid)</td>
<td>1.16±0.03 c</td>
</tr>
<tr>
<td>pH</td>
<td>4.61</td>
</tr>
</tbody>
</table>

1 LO and LB: conventional ovine and bovine yoghurt; LB-LWPCb and LB-LWPCo: yoghurts with bovine and ovine LWPC incorporation respectively.

a, b, c means within the same rows without the same superscript are significantly different (p<0.05).

Figure 2 represents the relationship between the syneresis index and yoghurt apparent viscosity of all tested products. The high amount of solids (15.5-16.0%) in tested formulations is in part, responsible for the low syneresis indexes observed (0.5-5.0%). However, the use of LWPC decreased water holding capacity by increasing yoghurts syneresis. This behaviour can be explained by the nature, proportion and incorporation form of proteins in each formulation (mainly caseins in conventional products and denaturated whey proteins in tested ones). Yoghurt viscosity varied inversely with syneresis, allowing the identification of three distinct groups of products. The first group includes ovine yoghurts (LO) and it is characterized by low values of syneresis and higher viscosity. Such behavior can be explained by their higher protein concentration and therefore the possibility of building a more cohesive polymer network. Set yoghurts prepared with LWPC (LB-LWPCo e LB-LWPCb) exhibited the lowest values in viscosity and the highest syneresis index. The last group includes conventional bovine yoghurts (LB) which present intermediate values for both parameters.
Fig. 2. Apparent viscosity (cP) vs syneresis index (%) for each type of yoghurt after 1, 14 and 21 days of storage

Although the total protein content between the last two groups of yoghurts (LB-LWPC and LB) does not differ significantly (Table 3), the protein’s nature proportion in the formulations (casein and whey proteins) was distinct (Table 1). As reported by Guzmán-González et al. (1999) these differences are responsible for the decrease in viscosity and increase in syneresis showed by yoghurts enriched with WPC with higher ratios of denatured whey proteins. Our results are also in accordance with data published by Modler et al. (1983) and Sodini et al. (2005) who concluded that products enriched with SMP or higher casein contents tend to produce more compact and, more viscous gels with higher retention capacity then the ones enriched with whey proteins. During storage, no particular trend in both properties was observed.

Despite the similarity in biochemical composition between LO and LB yoghurts the sensory panel differentiated both products (p<0.001). However, among LB based products no significative differences were found. The yoghurts with the incorporation of LWPC (LB-LWPCo and LB-LWPCb) were the preferred ones and the ovine yoghurts (LO) the less appreciated. These results showed that the yoghurts with closer textural properties, viscosity and syneresis to the conventional ones (LB) were more appreciated. The lack of familiarization with the ovine yoghurts by Portuguese consumers reflects the lower acceptance of these products.

4. Conclusion

It was concluded that partial substitution of conventional SMP by LWPC in set yoghurts (independently of their origin) is possible and can be very attractive not only concerning to the global process yield, as well as by reducing effluents and adding value to the existing products, but also in what concerns to their functional properties. Further work envisages the optimization of the LWPC denaturation step, as well as on the improvement of sensory properties of ovine milk yoghurts.
Acknowledgements

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