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ORIGINAL ARTICLE

Chemical characterisation and application of acid whey in fermented milk

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Abstract Acid whey is a by-product from cheese processing that can be employed in beverage formulations due to its high nutritional quality. The objective of the present work was to study the physicochemical characterisation of acid whey from *Petit Suisse*-type cheese production and use this by-product in the formulation of fermented milk, substituting water. In addition, a reduction in the fermentation period was tested. Both the final product and the acid whey were analysed considering physicochemical determinations, and the fermented milk was evaluated by means of sensory analysis, including multiple comparison and acceptance tests, as well as purchase intention. The results of the physicochemical analyses showed that whey which was produced during both winter and summer presented higher values of protein (1.22 and 0.97 %, w/v, respectively), but there were no differences in lactose content. During the autumn, the highest solid extract was found in whey (6.00 %, w/v), with larger amounts of lactose (4.73 %, w/v) and ash (0.83 %, w/v). When analysing the fermented milk produced with added acid whey, the acceptance test resulted in 90 % of acceptance; the purchase intention showed that 54 % of the consumers would 'certainly buy' and 38 % would 'probably buy' the product. Using acid whey in a fermented milk formulation was technically viable, allowing by-product value aggregation, avoiding discharge, lowering water consumption and shortening the fermentation period.

Keywords Cheese making · Beverages · Milk and dairy products · By-product

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Introduction

Milk whey is the liquid that remains from casein precipitation during cheese manufacturing (Magalhães et al. 2010). It is a by-product from this industry (Amante et al. 1999; Assadi et al. 2008; Baldissera et al. 2011; Khurana and Kanawjia 2007), which corresponds to between 70 and 90 % of total milk entering the process and it contains around 50 % of the nutrients from the raw material, including soluble protein, lactose, vitamins and minerals (Bylund 2003).

Milk whey composition depends on several factors, including the type of cheese being processed, the method of casein precipitation, milk thermal treatment, storage after milking, among others (Johansen et al. 2002; Lucas et al. 2006). Acid whey (pH <5.0) is generated in fresh-type cheese production, which includes acid coagulation of milk, cream or whey, or a combination of acid and rennet or acid and heating and it differs from sweet whey in terms of proteins, minerals and lactose concentrations. It also has higher acidity and calcium content, and the absence of caseinomacropeptide (Konrad et al. 2012). Some of the most popular cheeses in which acid whey is a by-product are, Cream Cheese, *Cottage*, *Quark* or *Tvorog*, *Fromage frais*, *Ricotta* and *Petit Suisse* (Schulz-Collins and Senge 2004).

Acid whey contains 4.2 to 4.9 % (w/v) of lactose, 93.5 % (w/v) of water, 0.55 to 0.75 % (w/v) of protein, 0.8 % (w/v) of ash and 0.04 % (w/v) of lipids (Bylund 2003). This type of whey commonly contains higher levels of ash and lower levels of protein, when compared with sweet whey, and its food application is limited, due to its acid and salty taste (Baldissera et al. 2011; Wong et al. 1978).

World production of milk whey is estimated at 180 to 190× 10^6 tonnes/year, with an yearly increase rate of 1-2 %, but only around 50 % is processed (Baldasso et al. 2011; Román et al. 2012). In 2011, in Brazil, around 680,000 tonnes of cheese were produced, corresponding to more than six million cubic meters of whey (Anuário 2012), which represented a loss of milk solids that went largely unused, and also represented a potential environmental threat (Cruz et al. 2009).

In the dairy market, fermented milk beverages are an important class of product that has grown at a rate six times higher than total dairy growth between 1998 and 2003; this behaviour appears to be related to increased consumer interest in functional foods (Baldissera et al. 2011; Granato et al. 2010; Khurana and Kanawjia 2007; Shibya and Mishrab 2013). As reported by Khurana and Kanawjia (2007), until 2003, Japan was the main market for this type of product, where the Yakult brand is the reference product for the entire category, and the other important markets were South Korea and Brazil. Data relating to the per capita consumption of milk beverages and fermented products including yoghurt is available for some countries, such as Germany, France and Denmark; in 2004 these values were 44.6, 28.9 and 21.9 kg/inhabitant, respectively (Khurana and Kanawjia 2007).

In Brazil, specific legislation for fermented milk (BRAZIL 2007) defines it as a product supplemented, or not, with other ingredients, obtained by the coagulation and decreased pH of milk, added, or not, of other dairy products, by lactic fermentation through the action of specific cultures of microorganisms, such as *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium* sp., *Streptococcus salivarius* subsp. *thermophilus* among other LAB. These specific bacteria should be viable and abundant in the final product. Milk whey, concentrated or not, is an allowed optional ingredient in the production of fermented milk under Brazilian legislation. Considering the high amounts of milk whey that are underutilised in animal feed, the present paper proposes using milk whey as a water substituent in fermented milk production, with a reduction in the fermentation period. If this liquid by-product was included in a food product as fermented milk, it would be possible to optimise the nutritional value of the final product, with complete consumption of the solids of the raw material and reduced risks of environmental pollution.

Materials and methods

Materials

This research was developed in association with a dairy factory in the central-eastern state of Paraná (IPARDES 2004), in the south of Brazil, with the donation of the acid whey and the other ingredients for producing the fermented milk, as well as access to processing equipment and facilities. Fresh acid whey was collected at the exit of a centrifuge (Westfalia KDB 30-02-076, Oelde, Germany) that separated it from the Quark mass in the processing of *Petit Suisse*-type cheese. The other ingredients added to the fermented base were pasteurised sucrose syrup (62° Brix), citric fruits aroma (559609–Firmenich, Cotia SP, Brazil), granulated potassium sorbate (Ntsac, Nantong-Jiangsu, China) and sweetened and concentrated orange juice (Orion, Palhoça SC, Brazil). All reagents were *pro analysis* grade and the equipment and materials were available at the State University of Ponta Grossa (UEPG).

Methods

Physicochemical characterisation of acid whey

The acid whey was collected immediately after its production once a week during 12 months, from November 2011 to November 2012 from a local dairy factory. Immediately after being collected, the samples were evaluated in the quality control laboratory of the dairy factory for protein content by employing the LECO® FP-528 (LECO Corporation, St. Joseph, MI, EUA) automatic system that is certified by the Association of Official Analytical Chemists (AOAC 1998). Ash content was assessed gravimetrically after complete incineration (muffle furnace at 550 °C/5 h). Total dry extract (drying oven at 105ºC/4 h) and total soluble solids were also quantified, the latter by a refractometer (Atago PAL-1, Saitama, Japan), as were lipids (butyrometer), lactose content (Lane-Eynon method) and acidity (expressed as lactic acid). All analyses were in accordance with Brazilian legislation, *Instrução Normativa* no. 68 (Brasil 2006).

Production of the fermented milk

Samples were produced in an industrial plant, using some equipment from the commercial process and other equipment from the quality control/research and development laboratories. Bovine milk (Holstein cows) pasteurised at 72–75 °C/15 s (VT20–GEA Sarstedt, Germany), skimmed and standardised at 0.03 % of lipids, and refrigerated to 5–7 °C (Inoxil model S38RKC, São Paulo SP, Brazil) was added to skimmed powder milk, vitamin mix (A, C, D, E) (Mix Cassab BTV–4353, Santo Amaro SP, Brazil) and sodium citrate (Cargill, Uberlandia MG, Brazil). The mix was thermally treated again (88–92 °C/5 min) and the temperature was lowered to 75 °C. After that, the mix was sent to fermentation tanks (Inoxil model S98.031.09, São Paulo SP, Brazil) and heated to 95 °C/3 h, a caramelisation stage, and was then cooled until 40 °C, when the appropriate starter culture containing *Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium* sp. was added. The process parameters tested in the present work were those in use in an industrial plant that processes fermented milk and which collaborated with this research. The fermentation period in use by the dairy industry was established as necessary for reaching the acidity value of 320 °D in the fermented base. In our study, fermentation at 43 °C proceeded for 25 and 40 h for the production of the fermented base.

For producing the fermented milk, the fermented base was taken from the fermentation tanks and refrigerated until it reached 5 ºC; it was then homogenised with a mixer and the other ingredients were added at the same amounts found in the commercial formulation: pasteurised sucrose syrup, sweetened concentrated orange juice, granulated potassium sorbate, citric fruit aroma and pasteurised acid whey (95 ºC/5 min). The final product was packed in milky coloured PET bottles and stored in a refrigerator at 7–10 °C.

The composition of the commercial, as well as the experimental fermented milk, is presented in Table 1.

Physicochemical characterisation of fermented milk with added acid whey

These analyses were carried out in the laboratories of the University. The protein content in the fermented milk was calculated from total nitrogen (N×6.25), as stated by the Kjeldahl method, total soluble solids by refractometry, lactose and reducing sugars by the Lane-Eynon method, and acidity was expressed as lactic acid, in accordance with Brazilian legislation, *Instrução Normativa* n° 68 (Brasil 2006). Glucose content was determined by the glucose oxidase (GOD) colorimetric method (Dahlquist 1961). The pH was measured by potentiometry (Micronal, model B474, São Paulo SP, Brazil) (IAL 2005).

Sensory analysis of the fermented milk with added acid whey

After having been approved by the Committee on Ethics in Research involving Humans, of the State University of Ponta Grossa (Process n°133.108/2012), the fermented milk samples, fermented for 25 and 40 h, were evaluated 2 days after production by the multiple comparison tests, according to NBR 13526 (ABNT 1995), including the commercial sample as standard, considering four repetitions. In this test, 13 trained tasters (three men and 10 women, aged between 26 and 35) were involved, and a basic scale was used for comparing experimental samples with the standard, with the extreme points identified as 'extremely worse' than the standard (1) and 'extremely better' than the standard (9). The evaluation form for the tasters contained a field for comments that they found necessary. The tasters were trained in the dairy factory by the Sensory Analysis and Innovation Team for monitoring products. The training programme included the following main tests: basic tastes, odour recognition, visual acuity with test kit colours, texture, viscosity and triangular tests. The samples (20 mL of refrigerated fermented milk) were codified and tasted randomly in transparent plastic cups, in quadruplicates, and the tests were distributed in four sessions over 3 days.

Table 1 Fermentation period and constituents of the commercial and experimental fermented milk

Parameter	Commercial	Experimental	
Fermentation period (h)	60	25	40
Fermented base (%)	25	25	25
Water (%)	54	0	0
Acid whey (%)	0	54	54
Other ingredients (%)	21	21	21

The acceptance test was carried out in the University 3 days after the production of the samples, with 77 consumers (40 % men and 60 % women) who evaluated the experimental product made with the 25 h fermented base. A nine-point hedonic scale with extremes identified as 'disliked very much' (1) and 'liked very much' (9) was used. The purchase intention was also evaluated in this test, with the same consumers, using a five-point scale, ranging from 'would certainly buy' (5) to 'would certainly not buy' (1) and employing the procedures described elsewhere (Meilgaard et al. 1991). The samples were presented in transparent plastic cups (20 mL of refrigerated product) and the analysis was performed in the sensory analysis laboratory, in individual booths with appropriate illumination at room temperature (20 °C), with non-trained tasters (students and employees of the University). The sensory evaluation forms contained a field for comments by the judges.

Statistical analysis

The physicochemical results were initially evaluated by the Levene test for verifying data distribution. After homogeneity was confirmed, the results were expressed as average±standard deviation and coefficient of variation. The Fisher test was used to compare the raw materials produced during different seasons. The Pearson correlation was employed for identifying possible associations between the parameters of the acid whey samples at $p<0.01$. The adopted significance level for the analyses was always $p<0.05$. The results of sensory analysis (multiple comparison tests) were submitted to ANOVA. The final product acceptability was calculated by average values of consumer responses and expressed as a percentage, and the purchase intention was shown as a histogram and as a percentage. The statistical analyses were carried out by using Action® software (Estacamp, São Carlos SP, Brazil).

Results and discussion

Physicochemical characterisation of the acid whey

Table 2 shows the results of the physicochemical analyses for the acid whey samples collected weekly from November 2011 to November 2012 in a Brazilian dairy factory.

Table 2 Physicochemical characterisation of acid whey samples (n=65)

	Acidity % (w/v)	Lipids % (w/v)	Ash % (w/v)	Water content % (w/v)	TSS % (w/v)	pH	Protein % (w/v)	Lactose % (w/v)
Average	0.61	0.09	0.61	94.44	5.57	4.37	0.84	4.18
SD	0.07	0.04	0.37	0.54	0.57	0.14	0.55	0.84
CV %	11.55	43.55	61.38	0.57	10.23	3.12	65.84	20.05

TSS total soluble solids, *SD* standard deviation, *CV* coefficient of variation

The average results were, as shown in Table 2, 0.84 % (w/v) of protein, 5.57 % (w/v) of TSS and acidity of 0.61 % (w/v, in lactic acid), close to those reported by Bylund (2003), which were between 0.55 and 0.75 % of protein, 6.2 to 6.5 % of TSS and acidity of 0.4 % (w/v, in lactic acid). Gallardo-Escamilla et al. (2005) found 0.76 % of protein and pH of 4.36 in whey from Quark cheese production. Turhan and Etzel (2004) reported 0.76 % (w/v) of protein for *Cottage* cheese whey, whereas Martínez-Hermosilla et al. (2000) reported, for whey of the same type of cheese, 0.75 % of protein, 6.32 % of TSS, 0.60 % of ash and 93.68 % of water content. Djuric et al. (2004) found 4.69 % of lactose, 0.82 % of protein and 0.50 % of ash in acid whey.

In previous research, our group analysed acid whey from Quark cheese production, collected in 2010 from the same dairy factory, and the results were pH of 4.20, acidity of 0.64 (% w/v, as lactic acid), total soluble solids of 5.2 % (w/v), protein of 0.58 % (w/v) and lipids of 0.04 % (w/v) (Barana et al. 2012).

The differences in chemical composition of this kind of by-product are related to several factors, including milking conditions, milk chemical composition and storage, as well as the cheese manufacturing proceedings (Johansen et al. 2002). Milk chemical composition is related to a cow's milking stage, cattle breed and nutrition, among other factors (Fagan et al. 2010; Glantz et al. 2009; Glantz et al. 2010; Johansen et al. 2002).

In the present study, the protein level was around 18 % higher than the values reported above, which might be related to the genetic improvement and high quality nutrition of the herds from the region where the samples of acid whey were collected. Ribas et al. (2004) studied the variations in total soluble solids of milk from 32,590 herds from Paraná, Santa Catarina and São Paulo states (all in the south of Brazil) and found an average protein content of 3.20 % (w/v), a little higher than those cited by Ribas et al. (2004), of 3.10 % (w/v), and by the Dairy Herd Improvement Analysis (DHIA) (Ribas et al. 2004) of 3.18 % (w/v).

The results of Pearson analysis revealed no correlation (p<0.01) between protein content and the other components of the acid whey, except for lactose with a negative correlation (r=-0.715) (Table 3).

Other significant correlations (p<0.01) were between water content and TSS (r=-0,974), lactose and TSS (r=-0.609), lactose and water content (r=-0.597), ash and water content (r=-0.420) and ash and TSS (r=0.411).

Table 3 Pearson correlation values for the physicochemical data of acid whey (n=65)

	Acidity	Lipids	Ash	Water cont	TSS	pH	Protein	Lactose
Acidity	1							
	p=—							
Lipids	−0.0664	1.0000						
	p=0.602	p=—						
Ash	−0.0357	0.1824	1.0000					
	p=0.780	p=0.149	p=—					
Water cont	0.0186	−0.2798	**−0.4201**	1.0000				
	p=0.884	p=0.025	**p=0.001**	p=—				
TSS	−0.0592	0.2790	**0.4110**	**−0.9735**	1.0000			
	p=0.642	p=0.026	**p=0.001**	**p=0.00**	p=—			
pH	0.0975	−0.2991	−0.1199	0.1539	−0.1430	1.0000		
	p=0.444	p=0.016	p=0.345	p=0.225	p=0.260	p=—		
Protein	−0.1897	−0.0511	0.0606	0.0405	−0.0540	−0.0502	1.0000	
	p=0.133	p=0.688	p=0.634	p=0.751	p=0.671	p=0.694	p=—	
Lactose	0.2090	0.1645	−0.0425	**−0.5971**	**0.6090**	0.0734	**−0.7158**	1.0000
	p=0.098	p=0.194	p=0.739	**p=0.000**	**p=0.000**	p=0.564	**p=0.000**	p=—

TSS total soluble solids

Table 4 presents the physicochemical composition of the acid whey samples from the four seasons. Ash content had a high coefficient of variation (61.38 %), with a major difference between summer and the other seasons. Other academic studies have reported that the mineral composition of liquid acid whey is very close to that of sweet whey, except for higher levels of calcium and magnesium, which may be attributed to the method of casein precipitation (Alsaed et al. 2013; Barana et al. 2012; Wong et al. 1978). As discussed by Jeličić et al. (2008), the calcium content in acid whey is higher, due to its higher solubility at low values of pH. As described by Lucas et al. (2006), in cheese production, the inorganic phosphorous is completely solubilised at pH 5.2, whereas calcium and magnesium solubilisation occurs only at lower values (pH 3.5). Thus, the higher the milk acidification before curd draining, the more minerals there will be in the whey. In the present study, process variations related to acidification may justify the differences that were found.

The values of lactose in the whey showed an average of 4.07 % for the warmer seasons and 4.28 % for the colder seasons. Silva (2004) reported that the different seasons had an impact on lactose content in cow milk; in warmer times it was lower when compared with colder periods, ranging from 4.5 to 5.0 % (w/v).

Our results for lipid content had high values of coefficient of variation, but lower than for protein content. It is important to consider that the milk used for producing the cheese that originated the acid whey for this research was skimmed milk. It must also be considered that milk skimming by centrifugation may vary, resulting in changes in the final content of lipids in the product. As Heck et al. (2009) have observed, of the main components of milk, lactose has the lowest level of variation, whilst lipids are the component with the highest level of variation; protein has an intermediary level of variation. In the present study, lactose had a low variation, whereas protein presented a higher coefficient of variation.

The highest protein contents were found in the samples from summer and winter. Johansen, Vegarud and Skeie (2002) studied the chemical composition of Cheddar and Novergia cheese whey in different seasons and reported similar results, with higher concentrations of β-lactoglobulin in summer and winter than in spring; α-lactalbumin concentrations were higher during summer than in spring and autumn. Those differences are attributed to weather interference in the quality and availability of forage. During winter, the animals receive nutritional supplementation to overcome shortage of fodder. Heck et al. (2009) analysed milk samples from the Netherlands for a period of 1 year and detected that both protein and lipids levels were maximum in the winter when animal feeding includes around 30 % more concentrate, which is rich in starch, in relation to fodder, which is rich in fibre. This starch-rich feed increases the production of propionic acid in the rumen and it is the main glucose precursor, resulting in hormonal signs in cows and increasing milk protein concentration (Heck et al. 2009), which is in accordance with the results found in the present study.

Multiple comparison tests and physicochemical characterisation of the fermented milk

Table 5 presents the physicochemical characterisation of fermented milk samples prepared with fermented bases of 25 and 40 h. The acid whey used as water substituent in preparing the fermented milks had a protein content of 0.488 % (w/v). It is possible to note that the fermented milk made with the less fermented base (25 h) presented a higher concentration of lactose, lower acidity and higher pH value when compared with the product made with base fermented for 40 h, as expected. Although lactose is only mildly sweet (Holsinger et al. 1974), its excess influenced the sweetness of the final product. However, this is easy to correct by reducing the amount of other ingredients in the formulation, i.e. sucrose syrup.

Table 4 Physicochemical characterisation of acid whey collected over the four seasons, from November 2011 to November 2012

	Acidity % (w/v)	Lipids % (w/v)	Ash % (w/v)	Water content % (w/v)	TSS % (w/v)	pH	Protein % (w/v)	Lactose % (w/v)
Spring (Sep/Dec)	0.596^{ab}	0.085^{a}	0.619^{a}	94.571^{a}	5.425^{bc}	4.312^{b}	0.515^{c}	4.216^{ab}
SD	0.03	0.03	0.21	0.38	0.38	0.13	0.23	0.42
Summer (Dec/Mar)	0.638^{a}	0.061^{b}	0.367^{b}	94.837^{a}	5.129^{c}	4.478^{a}	0.968^{ab}	3.942^{b}
SD	0.06	0.06	0.13	0.68	0.69	0.06	0.31	1.05
Autumn (Mar/Jun)	0.615^{ab}	0.100^{a}	0.828^{a}	94.066^{b}	6.001^{a}	4.441^{a}	0.639^{bc}	4.734^{a}
SD	0.12	0.00	0.60	0.27	0.33	0.13	0.39	0.64
Winter (Jun/Sep)	0.580^{b}	0.100^{a}	0.661^{a}	94.230^{b}	5.770^{ab}	4.227^{c}	1.218^{a}	3.840^{b}
SD	0.04	0.00	0.19	0.34	0.34	0.04	0.84	0.80

Means followed by different letters in the columns differ statistically ($p<0.05$)–Fisher test

TSS total soluble solids, *SD* standard deviation. Summer: $n=18$, Winter: $n=16$, Autumn: $n=16$, Spring: $n=15$

Table 5 Physicochemical analysis of fermented milk samples produced with bases fermented for 25 and 40 h

Fermentation period (h)	Base volume % (w/v)	Protein % (w/v)	°Brix	Acidity °D	pH	Glucose % (w/v)	Lactose % (w/v)	Reducing sugars % (w/v)*
25	100	2.54	22	90	4.17	1.10	7.44	8.54
SD	–	0.07	–	3.0	0.06	0.16	0.36	–
40	100	2.06	22	100	4.06	1.91	6.29	8.20
SD	–	0.08	–	4.3	0.05	0.06	0.65	-

SD standard deviation (n=3); * Reducing sugars=glucose+lactose

The sensory analysis showed that no difference was detected between the products made with bases fermented for 25 or 40 h (p>0.05), even though it was expected that the fermentation period would influence the development of aroma compounds. The physicochemical analyses showed some differences, which may be explained by slight variations in processes and raw materials.

The aroma and flavour development in fermented milk is a complex process involving several biochemical pathways that are catalysed by microbial enzymes with protein hydrolysis, which results in the sensory evolution of the product (Smit et al. 2005). Fermented milk production includes probiotic lactic acid bacteria, mainly *Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium* sp. and prolonged incubation times are necessary (Mohammadi et al. 2012). It has been reported that *Lactobacillus acidophilus* is responsible for the production of volatile aroma compounds, including acetaldehyde, diacetyl and acetoin (Imhof and Bosset 1994). *Bifidobacterium* sp. contributes to aroma by producing ethanol, diacetyl, acetaldehyde, propanon and butanon, whereas *Lactobacillus casei* produces ethanol, diacetyl and acetoin (Imhof and Bosset 1994). Consequently, a reduced fermentation time would be detrimental to the sensory quality of the final product but, in the present work, this was not noted and this might be related to the fact that the product formulation included sucrose syrup, citric fruit aroma and orange juice. The studied product was flavoured, which masks the aroma generated during base fermentation. It should also be considered that the final product had 54 % (v/v) of acid whey in substitution to water and that this fermented dairy by-product contains aroma compounds.

There are relatively few academic studies showing the influence of milk whey in the flavour and aroma composition of food products to which it is added. The flavour of whey solids has always been considered a limiting factor in its food use (Mortenson et al. 2008). Gallardo-Escamilla et al. (2005) compared whey samples from different cheese processing conditions and they reported that the sensory evaluation showed that acid whey from Quark cheese had better ratings for yoghurt taste and flavour, when compared with samples of whey from other types of cheeses.

In the multiple comparison tests, the average values attributed were 2.77 and 2.48 for the fermented milk, with 25 and 40 h fermentation, respectively, allowing the conclusion that both products were graded as 'very worse' and 'moderately worse' than the standard. No sample was evaluated as 'better than the standard' by the trained sensory team.

The commercial fermented milk, considered as the reference in the present work, presented pH between 3.8 and 4.0, 18° brix and acidity ranging from 70 to 75°D. It is clear that the experimental products, both produced with 25 and 40 h fermented base and added acid whey, were sweeter and more acid than the standard sample, corroborating the comments of the tasters who reported disliking samples due to an excess of sweet and/or acid taste.

The Brazilian Association of Consumer Rights (ProTeste 2011) studied eleven commercial brands of fermented skim milk samples, asking consumers to evaluate aroma and consistency, and the results showed that the commercial sample considered as standard in the present study had one of the best acceptance levels. The commercial samples presented differences in the levels of sugar, acidity and protein, and it was reported that consumer acceptance also had an important influence on the price of products. Our results from the multiple comparison tests do not allow the conclusion that the experimental fermented milk would have a low acceptance level because the standard sample was evaluated as one of the best among all of those available in the Brazilian market.

In a previous paper, Barana et al. (2012) tested acid whey from the same processing plant used in the present study in order to produce another type of fermented dairy beverage and they found sensory evaluation results that indicated significant differences in relation to a standard beverage made without acid whey. The beverages with added acid whey to replace water received the best ratings in the sensory evaluation and the authors concluded that this by-product could be an economical option for incorporating into that kind of fermented product. Barana et al. (2012) used acid whey that corresponded to 20 % of the volume of milk drink, whereas in the present study, the same by-product was used in considerably larger amounts, totalling 54 % of the volume of the final product.

Table 6 Physicochemical analysis of fermented milk produced with base fermented for 25 h

Fermentation period (h)	Protein % (w/v)	°Brix	Acidity °D	pH	Lactose % (w/v)	Glucose % (w/v)	Reducing sugars % (w/v)*
25	2.0	22	95	4.10	6.07	1.50	7.57
SD	0.06	–	4.0	0.05	0.88	0.07	–

SD standard deviation (n=3); * Reducing sugars=glucose+lactose

Acceptance test and physicochemical characterisation of the fermented milk

Considering the results of the multiple comparison tests that did not differentiate the samples, the fermented milk produced with base fermented for 25 h was selected to be evaluated by the acceptance test. The physicochemical characteristics of a new batch of the product are shown in Table 6. The acid whey used for producing this sample had a protein content of 0.48 % (w/v), pH 4.12 and 4.52 % (w/v) of lactose.

The results showed that the reducing sugar content was of 7.57 % (w/v). Santos et al. (2008) produced dairy beverages substituting milk by whey in proportions of 20, 40, 60 and 80 % and they detected reducing sugars in concentrations of 7.74, 8.81, 10.48 and 10.57 % (w/v), respectively. In the present study, sucrose syrup was added to the formulation of the fermented milk and it is well known that this disaccharide hydrolyses easily, resulting in a mixture of the reducing sugars, glucose and fructose (Konkel et al. 2004).

Cunha et al. (2008) produced fermented milk with 70 % of milk and 30 % of whey, and found 2.23 % of protein, 72.33°D of acidity, pH 4.53 and 13.29 % (w/v) of total sugars. As the milk was partially replaced by whey, the product had a lower total solids content when compared with the fermented milk made exclusively with milk. Almeida et al. (2001) found 1.94, 1.97 and 2.12 % (w/v) of protein in dairy beverages with added whey, in concentrations of 50, 40 and 30 %, respectively. Another work reported on fermented dairy beverage produced with the addition of 10, 30 and 50 % of whey to replace the milk, which had 2.08, 2.02 and 1.65 % (w/v) of protein (Oliveira et al. 2006). It is important to highlight that those works considered whey as a substitute for milk, differently from our research, which used a fermented base (added skim powder milk, as described earlier) and the whey replaced only water from the formulation.

The results of the acceptance test in the present study showed 90 % acceptance, with an average response of 8.10, which corresponded to 'liked very much' on the hedonic scale. As this part of the sensory analysis was carried out inside the University campus, the consumers were students, professors and employees; 52 % were younger than 25, 30 % were between 25 and 30, 13 % were between 36 and 50 and 5 % were over 50 years old.

As shown in Fig. 1, 43 % of consumers 'liked very much', 38 % 'liked extremely' and 13 % 'liked moderately'. The other categories of the scale were not mentioned by the consumers in this test.

Santos et al. (2008) studied milk replacement by whey in concentrations of 20, 40, 60 and 80 % in the production of fermented dairy beverage. They concluded that 40 % was the best level of milk substitution, with an average sensory acceptance value of 7.8, close to 'liked very much' on the hedonic scale.

In general, the low preference for beverages containing whey is related to the lower viscosities of these products. The

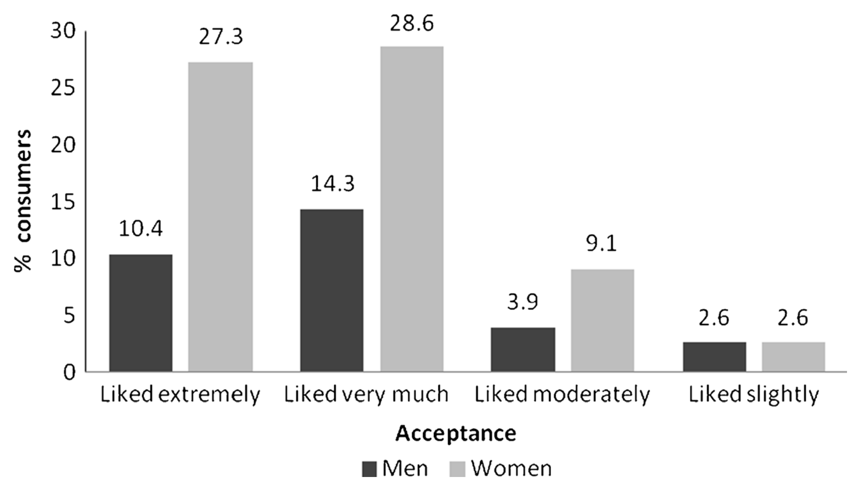

Fig. 1 Histogram of acceptance test of the fermented milk by consumers, considering gender

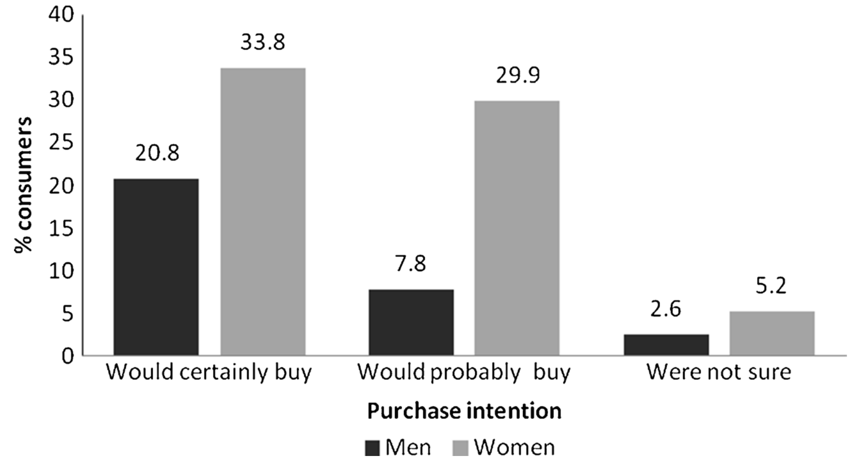

Fig. 2 Histogram of purchase intention by gender

consistency of dairy products is linked with casein gel formation and this protein is scarce or absent in whey (Santos et al. 2008). On the other hand, in our work the fermented milk that was produced was a very fluid beverage and the whey was replacing water and not milk in the formulation, differently from many other studies in this field. Replacing water by acid whey resulted in a more consistent product and some consumers noted in their sensory evaluation forms that the experimental fermented milk was more 'full-bodied'. In fact, the acceptance level in the present study was high, if compared with other published data.

It was possible to check by the sensory evaluation forms that the youngest consumers (aged under 25) attributed more 9 ratings ('liked extremely'), and, in general, were the class of consumers that considered the high sweetness level to be positive. From the male consumers that participated in this sensory analysis, 46 % 'liked very much' and 40 % of female consumers 'liked extremely'.

Among the comments by consumers in the sensory evaluation forms, most related to the product's sweetness, and those consumers that 'liked extremely' the product emphasised that this was a favourable aspect. On the other hand, for the consumers that 'liked moderately' the product, the sweetness was excessive and unfavourable. In a Brazilian market study, the commercial sample of fermented milk with the lowest sugar content had the worst sensory acceptance (ProTeste 2011). Other works also report the preference of Brazilian consumers for sweeter beverages, mainly among young people (Nogueira et al. 2007).

Figure 2 shows the results for purchase intention; around 54 % of consumers that were included in this sensory evaluation 'would certainly' buy the product, 38 % 'would probably' buy and only 8 % 'were not sure' about purchasing. No negative scale category was indicated by the consumers in this test.

All the sensory analysis results were favourable and allowed the consideration of the commercial use of acid whey, as well as reducing fermentation time in the production of this type of fermented milk.

For the dairy factory that donated the acid whey used in this study, using acid whey commercially in fermented milk production could bring considerable gains, including the reduction of water consumption by around 180 m^3 monthly, the production of a more consistent and nutritive product, the reduction of costs of whey transportation, and the reduction of sucrose syrup use (to be tested) due to enhanced sweetness from lactose present in the whey. The process would also be quicker as base fermentation could be concluded in 25 h.

Conclusion

The results of acid whey chemical composition showed homogeneity, and only the level of protein was a little higher than the levels reported in similar works. The coefficient of variation of the whey constituents were in accordance with the literature, with higher values for protein (65.84 %), ash (61.38 %) and lipids (43.55 %). The differences in the composition of acid whey during the seasons revealed higher contents of protein in winter (1.22 %, w/v) and summer (0.97 %, w/v). Significant differences were found in different seasons for all constituents, except for lactose. The highest total soluble solids concentration (6.00 %, w/v) was found in the whey produced in the autumn.

Considering the issue of industrial processing, it was possible to completely replace water by the acid whey, as well as to have a fermentation time of 25 h. The final product had protein and acidity levels that were in accordance with Brazilian legislation, and nutritional enrichment with whey solids.

Liquid acid whey was a viable ingredient for producing the fermented milk but there are several other opportunities for this nutritive and abundant dairy by-product.

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