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Fermentation of whey-derived matrices by *Kluyveromyces marxianus*: alcoholic beverage development from whey and fruit juice mixes

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Research Article

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Abstract

This research paper addresses the hypotheses that *Kluyveromyces marxianus* can be cultured with good alcohol production on different whey-derived matrices, and that the fermented product can be used in order to develop alcoholic beverages with acceptable sensory characteristics by mixtures with yeast-fermented fruit-based matrices. Growth and fermentative characteristics of *Kluyveromyces marxianus* LFIQK1 in different whey-derived matrices were explored by culturing (24 h, 30 °C) on reconstituted whey, demineralized whey, heat-treated whey and milk permeate media. High lactose consumption, ethanol production and yield were observed. Reconstituted whey matrix was selected for mixing with orange or strawberry juices fermented using *Saccharomyces cerevisiae* to obtain alcoholic beverages (W-OR and W-ST, respectively). Consumer evaluation of beverages was performed using acceptability and Check-All-That-Apply (CATA) questions. Good acceptance was observed, significantly higher for W-ST than for W-OR. CATA questions gave information about organoleptic characteristics of beverages. Penalty analysis showed W-R and W-ST were positively associated with smooth/refreshing and fruity/natural, respectively. Liking was represented, accordingly with penalty analysis, by natural/refreshing. A novel alternative for utilization of whey and whey-related matrices by alcoholic beverages production with natural ingredients is presented.

Whey can be regarded as the aqueous fraction of milk, accounting for around 55% of milk nutrients (Panesar and Kennedy, 2012) and thus having high organic matter content; mainly lactose (70–72% of total solids), whey proteins, vitamins and salts (Arshad *et al.*, 2023). Due to its high organic matter content, whey has high contaminating potential, and inappropriate disposal may pose significant environmental impact (Guimarães *et al.*, 2010). A significant proportion of the whey produced worldwide is not properly treated or industrialized, still being discarded as an effluent, and the search for new alternatives for whey reuse remains as an active research field (Arshad *et al.*, 2023).

The presence of lactose and other nutrients for microbial growth makes whey one of the most potent raw materials for the biotechnological production of different high-added value bio-products (Panesar and Kennedy, 2012). Among yeasts, only 2% of known species possess lactose fermenting capacity (Fonseca *et al.*, 2008). *Kluyveromyces marxianus*, commonly isolated from dairy products, stands out for its high lactose assimilating capacity and thermotolerance (Lane and Morrissey, 2010). One of the applications previously described for *K. marxianus* is the use for ethanol production in whey or whey-derived media, for utilization as a biofuel (Sansonetti *et al.*, 2009). However, the fact that *K. marxianus* possesses GRAS (generally regarded as safe) and QPS (qualified presumption of safety) status means that its application for fermented food production is also feasible (Lane and Morrissey, 2010).

Beverage production is one of the simplest and most profitable options for whey utilization in human nutrition (Panesar and Kennedy, 2012; Barukčić *et al.*, 2019). However, due to the relatively high lactose:glucose ratio and acid taste (especially for acid whey) its flavour is usually perceived as unpleasant when consumed directly, and improvement of sensory characteristics is required (Fagnani *et al.*, 2018; Yamahata *et al.*, 2020). Combination with fruit juices is an interesting option for improving sensory quality of whey-based beverages (Janiaski *et al.*, 2016; Fagnani *et al.*, 2018). Another alternative is the production of fermented beverages from whey. There is previous research based on lactic acid bacteria or mixed lactic–alcoholic fermentation (Pescuma *et al.*, 2010; Kadyan *et al.*, 2021). Respecting the use of yeasts only, Kosikowski and Wzorek (1977) obtained a ready-to-drink alcoholic beverage using pure

cultures of *K. marxianus* to ferment whey or whey permeate powder solutions. Recently, Yamahata *et al.* (2020) used a similar strategy to produce an alcoholic beverage from whey or partially demineralized whey powder solutions reconstituted at different concentrations and fermented by *K. marxianus* NBRC 1735.

According to previous scientific research, alcoholic beverages produced using whey or whey-derived matrices as starting material for *K. marxianus* fermentation need improvements from the sensory point of view, and hence there is a need for further development in whey-based alcoholic beverages research field. As far as we know, there are no scientific reports where alcoholic whey-based beverages are produced by mixing fermented whey with fermented fruit juices.

The aims of our work were: (i) to explore growth and fermentative characteristics of *Kluyveromyces marxianus* LFIQK1 in different whey and whey-derived matrices, (ii) to develop alcoholic beverages by mixing one of these fermented whey-based matrices with different fruit juices, previously fermented using a commercial *Saccharomyces cerevisiae* strain, and finally (iii) to carry out a sensory evaluation based on consumers using Acceptability and Check-All-That-Apply (CATA) questions, for characterization of the alcoholic beverages obtained.

Materials and methods

Ingredients and chemical compounds

Whey powder and 45% demineralized whey powder were provided by Lácteos La Cristina (Villa María, Córdoba, Argentina). Lactose monohydrate, anhydrous glucose, sodium chloride and ethanol (pro-analysis) were purchased from Cicarelli (Laboratorios Cicarelli, San Lorenzo, Santa Fe, Argentina). Yeast extract and meat peptone were purchased from Biokar (Biokar Diagnostics, Beauvais, France).

Yeast strains

For lactose fermentation in whey and whey-derived matrices, a yeast strain of *Kluyveromyces* genus, originally isolated from kefir and belonging to the collection of the Food Technology and Biotechnology Department at Facultad de Ingeniería Química (Universidad Nacional del Litoral, Santa Fe, Argentina) was selected. The strain, identified as *Kluyveromyces marxianus* by molecular techniques at the Mycology and Molecular Diagnostics Laboratory (Universidad Nacional del Litoral, Santa Fe, Argentina), was named *Kluyveromyces marxianus* LFIQK1. For fermentation of fruit juices, commercial *Saccharomyces cerevisiae* SafAle S-04 (Fermentis Ltd., Marcq-en-Baroeul, France) was selected, frequently used in beer brewing with good growth and ethanol production reported when fermenting glucose, fructose and sucrose (Comelli *et al.*, 2016).

Microbiological and physicochemical analyses

Biomass was determined as described by Zafar and Owais (2006) with some modifications, as described in online Supplementary File. For mould and yeast counts determination, colonies were counted on moulds and yeast agar media plates after incubation under aerobic conditions (28 °C, 5 d), final results expressed as CFU/ml.

For total sugar content estimation in fruits or fruit juices, soluble solids (SS) determinations were made with a manual refractometer (ZGRB-32ATC) and expressed as Brix degrees (°Bx). pH-values were obtained using Horiba LAQUAtwin pH-meter (Horiba Ltd., Kyoto, Japan). For ethanol determination, a commercial enzymatic-spectrophotometric assay kit (Ethyl alcohol, Roche Cobas) was adapted, and for lactose determination, a commercial kit for enzymatic-colorimetric glucose quantification (Wiener Lab, Rosario, Argentina) was employed, both methods described in the online Supplementary File. For all spectrophotometric measurements a Genesys 10S UV-VIS (Thermo Scientific, Germany) equipment was used.

Growth and fermentative characteristics of Kluyveromyces marxianus LFIQK1 in different whey and whey-derived matrices

Growth, lactose consumption and ethanol production by *K. marxianus* LFIQK1 was assessed on four matrices: reconstituted whey (W), reconstituted demineralized whey (DW), heat-treated reconstituted whey (HW) and milk permeate (PER). For W and HW media, solutions were prepared from whey powder to 65 g/l (typical solids level of cheese whey) by adding 26 g of powder to 500-ml borosilicate glass bottles containing 400 ml of sterile distilled water. After homogenization, W solutions were stored at 2 °C until the next day, while HW solutions were steam sterilized in autoclave (15 min, 121 °C) and centrifuged (10 min, 1000 g) for heat-denaturized whey proteins removal. DW was prepared as W medium, but using demineralized whey powder as starting material. A fourth medium assayed, milk permeate (PER), was obtained from whole milk by ultrafiltration (FIQ3001 membrane equipment, Hidrobiot, Santo Tomé, Argentina) with a 10 kDa molecular weight cut-off Koch HFK-131 membrane (Koch Membrane Systems, Wilmington, MA, USA) and kept at −18 °C until use. For each medium, laboratory-scale fermentations were performed in duplicate.

Pure *K. marxianus* LFIQK1 was first cultured on YPL broth, containing yeast extract, meat peptone and lactose, then subdivided in portions, centrifuged and cells resuspended in 0.9% sterile sodium chloride solution, that were added as inoculum to corresponding media in borosilicate glass bottles (further details given in the online Supplementary File). After homogenization, bottles sealed with rubber stoppers were cultured (24 h, 30 °C) in static conditions (online Supplementary Fig. S1). Initial concentration of *K. marxianus* in all fermented media was 2.5×10^7 CFU/ml. Samples were taken at 0, 8, 16 and 24 h, a portion immediately processed for biomass and pH determination, the remaining stored at −18 °C for ethanol and lactose determination.

Development of alcoholic beverages obtained by fermentation from whey and fruit juices

The alcoholic beverages were obtained by mixing fruit media (orange juice or strawberry pulp) fermented by *S. cerevisiae* SafAle S-04 and whey, fermented by *K. marxianus* LFIQK1. Complete description given in the online Supplementary File. The three different fermentation media employed were:

i. Whey medium (W): prepared from whey powder as described previously. Whey solutions were stored at 2 °C until fermentation the following day.

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ii. Orange medium (OR): Oranges (*Citrus sinensis*) juice. Initial pH and SS values were 3.6 ± 0.1 and 12.9 ± 0.2°Bx, respectively.
iii. Strawberry medium (ST): Strawberries (*Fragaria* × *ananassa*) pulp. Initial pH and SS values were 3.4 ± 0.1 and 8.8 ± 0.0°Bx, respectively.

For whey fermentation, preparation of starters and fermenting conditions by *K. marxianus* LFIQK1 were the same as described previously. For fruit juices fermentation, inoculum preparation steps were the same as for whey fermentation, but using pure *S. cerevisiae* SafAle S-04 on malt extract agar (MEA) slant that was then propagated in sterile YPD broth (0.5% (w/v) yeast extract, 1% (w/v) meat peptone and 4% (w/v) dextrose). Inocula were finally added to juices in each plastic bottle and after homogenization, bottles sealed with rubber stoppers equipped with an airlock were incubated statically (48 h, 18 °C).

After fermentation, media were centrifuged (10 min, 1000 g), and supernatants poured into plastic bottles for beverage preparation. A total amount of 2.11 kg of fermented orange juice and 1.37 kg of fermented strawberry juice were obtained after centrifugation, thus overall yields (fermented juice obtained/fruit processed) were 29.6 and 31.8%, respectively. Beverages were prepared by 1:1 mixing of fermented whey with fermented orange (W-OR beverage) or fermented strawberry juice (W-ST beverage), and commercial sucrose addition (4% w/v in both cases) for taste improvement (online Supplementary Fig. S2). Beverages were immediately sampled for yeast and moulds count, pH, SS and ethanol determination, and stored at 2 °C until the next day for sensory analysis.

Sensory evaluation of the alcoholic beverages obtained by fermentation from whey and fruit juices

A total of 101 consumers evaluated the fermented beverages samples using overall liking hedonic scale and Check-All-That-Apply questions. Testing took place in a sensory laboratory in individual sensory booths, designed in accordance with ISO 8589:2007 and after Ethics Committee of Universidad Nacional del Litoral approval. For each beverage, consumers were asked to test a first time and then rate overall liking using a 9 points hedonic scale. After rating overall liking of each sample, consumers were asked to re-taste samples and complete a CATA question with 24 terms related to sensory characteristics of fermented beverages. Consumers were asked to check all the terms they considered appropriate to describe each beverage. Terms were selected based on published data (Ares *et al.*, 2015; Farah *et al.*, 2017) and considering the attributes selected by trained assessors in preliminary studies. The complete description of the sensory evaluation performed is provided in the online Supplementary File.

Statistical analysis

Two-way ANOVA with fixed factors (fermenting medium and fermenting time) and interaction was performed to evaluate

Table 1. Average values and standard deviations corresponding to reconstituted whey (W), reconstituted demineralized whey (DW), heat-treated reconstituted whey (HW) and milk permeate (PER) media during fermentation with *Kluyveromyces marxianus* LFIQK1

Medium	Time (h)	Biomass (g/l)	pH	Lactose (g/l)	Ethanol (g/l)
W	0	0.43 ± 0.03^{b}	6.3 ± 0.0^{c}	$43.5 \pm 1.1^{A\gamma}$	ND
	8	1.23 ± 0.00^{de}	5.6 ± 0.0^{f}	$41.0 \pm 1.5^{A\beta}$	4.1 ± 0.4^{ab}
	16	2.3 ± 0.2^{h}	5.1 ± 0.0^{h}	$13.5 \pm 1.2^{A\alpha}$	12.4 ± 1.3^{e}
	24	2.91 ± 0.03^{j}	4.7 ± 0.1^{j}	$11.9 \pm 1.2^{A\alpha}$	17.0 ± 0.7^{f}
DW	0	0.29 ± 0.01^{ab}	6.8 ± 0.1^{b}	$53.5 \pm 1.6^{C\gamma}$	ND
	8	1.07 ± 0.01^{cd}	5.8 ± 0.1^{e}	$47.5 \pm 3.1^{C\beta}$	3.6 ± 0.4^{ab}
	16	2.08 ± 0.06^{g}	5.3 ± 0.0^{g}	$14.6 \pm 1.9^{C\alpha}$	9.6 ± 0.5^{d}
	24	2.59 ± 0.04^{i}	5.0 ± 0.1^{i}	$12.5 \pm 0.0^{C\alpha}$	17.6 ± 0.5^{f}
HW	0	0.29 ± 0.06^{ab}	6.0 ± 0.0^{d}	$50.2 \pm 0.8^{AB\gamma}$	ND
	8	1.53 ± 0.04^{f}	5.4 ± 0.0^{g}	$40.5 \pm 2.7^{AB\beta}$	2.5 ± 0.2^{a}
	16	2.22 ± 0.03^{gh}	5.3 ± 0.0^{g}	$14.8 \pm 1.5^{AB\alpha}$	6.8 ± 1.3^{c}
	24	2.70 ± 0.02^{i}	4.8 ± 0.1^{j}	$12.7 \pm 0.2^{AB\alpha}$	13.0 ± 1.4^{e}
PER	0	0.20 ± 0.03^{a}	6.9 ± 0.0^{a}	$48.4 \pm 2.8^{BC\gamma}$	ND
	8	0.5 ± 0.2^{b}	5.8 ± 0.1^{e}	$42.8 \pm 6.1^{BC\beta}$	4.2 ± 0.0^{b}
	16	0.87 ± 0.01^{c}	5.7 ± 0.0^{ef}	$15.6 \pm 2.0^{BC\alpha}$	12.3 ± 0.6^{e}
	24	1.3 ± 0.1^{e}	5.4 ± 0.0^{g}	$12.9 \pm 0.4^{BC\alpha}$	16.3 ± 0.1^{f}
Culture medium		*	*	*	*
Culture time		*	*	*	*
Interaction		*	*	**NS**	*

ND, Non detectable; NS, No significant effect ($P > 0.05$), *: Significant effect ($P < 0.05$).
Last rows show the ANOVA result for the different factors analysed.
$^{a-j}$: Average values in the same column with different superscript letters are significantly different ($P < 0.05$) for Biomass, pH and Ethanol determinations.
$^{A-C}$: Average values in Lactose column with different superscript letters are significantly different ($P < 0.05$) for Medium factor.
$^{\alpha-\gamma}$: Average values in Lactose column with different superscript letters are significantly different ($P < 0.05$) for Time factor.

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Table 2. Final soluble solids, pH and ethanol values for orange juice (OR), strawberry pulp (ST), whey (W) media and beverages prepared by mixing of fermented whey with fermented orange juice (W-OR) or fermented strawberry juice (W-ST)

Medium/beverage	pH	SS (°Bx)	Ethanol (g/l)
ST	3.2 ± 0.0^{a}	3.6 ± 0.0^{b}	24.6 ± 0.6^{b}
OR	3.4 ± 0.1^{ab}	6.9 ± 0.0^{d}	38.3 ± 2.3^{d}
W	4.8 ± 0.1^{d}	3.3 ± 0.1^{a}	15.8 ± 1.5^{a}
W-ST	3.6 ± 0.1^{bc}	6.6 ± 0.0^{c}	16.6 ± 0.8^{a}
W-OR	3.7 ± 0.0^{c}	8.5 ± 0.0^{e}	28.1 ± 0.2^{c}

$^{a-e}$: Average values in the same column with different superscript letters are significantly different ($P < 0.05$)

differences in biomass, pH, lactose and ethanol values when evaluating growth and fermentative characteristics of *K. marxianus* LFIQK1 in different whey and whey-derived matrices (Statgraphics Inc., Rockville, MD, USA). One-way ANOVA with fixed factor was carry out for overall liking, pH, soluble solids and ethanol in alcoholic beverages development experience. When differences between treatment effects were significant ($P < 0.05$), multiple comparison of means was carried out using Tukey test (Statgraphics Inc., Rockville, MD, USA). Cochran's *Q* test (Manoukian, 1986) was carried out to identify significant differences among samples for each of the sensory terms considered on CATA questions (XLSTAT 2014, Addinsoft). In order to link information from CATA question with acceptability, penalty analysis was performed by calculating, for each evaluated product, its average acceptability across all consumers that evoked (checked) the attribute in the CATA question and all that did not. The difference between these two values can be considered an estimation of how much product acceptability changes when attribute is present or not (Varela and Ares, 2014).

Results and discussion

Growth and fermentative characteristics of Kluyveromyces marxianus *LFIQK1 in different whey and whey-derived matrices*

In order to explore growth and fermentative characteristics of *K. marxianus* LFIQK1, values for biomass, pH, lactose and ethanol were obtained in samples taken at 0, 8, 16 and 24-h culture (Table 1).

Evolution of biomass values showed that *K. marxianus* LFIQK1 grew in all media assayed. After 24 h, yeast growth for W, DW and HW media reached final values of between 2.5 and 3.0 g/l, while for PER medium it was around 1.3 g/l. This difference may be explained by considering the permeate composition, which is poorer in nutrients than the other matrices, because of whey protein removal by ultrafiltration. Lower yeast counts and biomass values (respect to those observed in whey) have already been reported in previous research employing other protein-reduced whey-derived media such as scotta (Zoppellari and Bardi, 2013) or both scotta and whey permeate (Sansonetti *et al.*, 2009). Total decrease for pH values was moderate in all cases (1.2–1.8 pH units), and final pH values were 4.7 ± 0.1, 5.0 ± 0.1, 4.8 ± 0.1 y 5.4 ± 0.0 for W, DW, HW and PER, respectively.

K. marxianus LFIQK1 strain showed an important lactose-fermenting activity (Table 1). In all cases, most of the lactose initially present was consumed between 8–16 h after the onset of the experiment. Despite different initial levels, no significant differences were observed in final lactose concentration (between 11 and 13 g/l), and more than 70% of initial lactose was consumed in all cases. These performances may be improved, and virtually all lactose removed with longer fermentation times or mechanical agitation (Ozmihci and Kargi, 2007).

As a consequence of yeast fermentation, ethanol was present in significant amounts at the end of the experiment in all matrices assayed (Table 1). Final ethanol values were similar (16–18 g/l) for W, DW and PER, while significantly lower for HW (13.0 ±

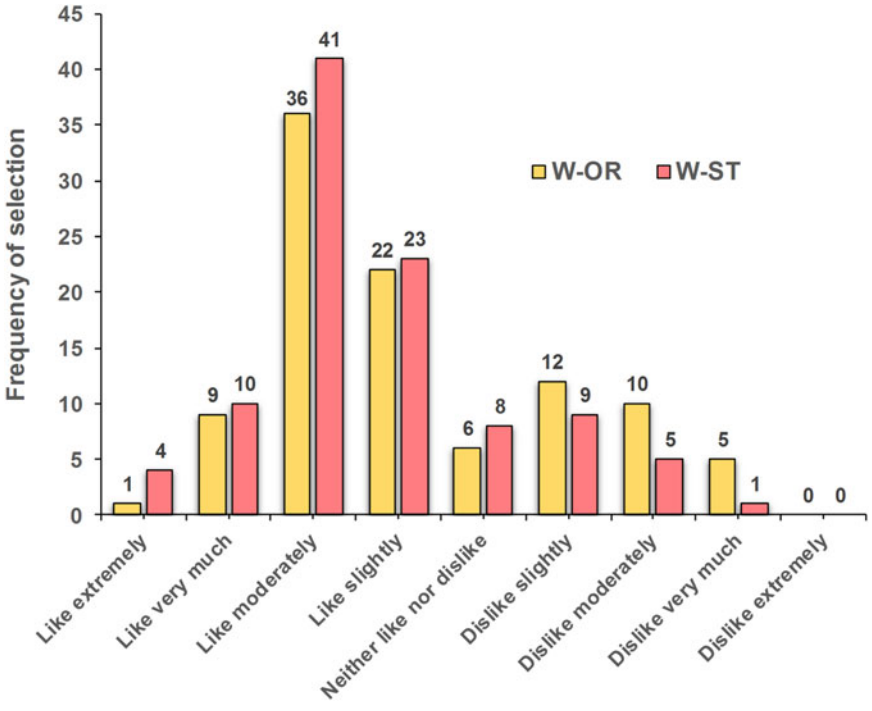

Figure 1. Nine-point hedonic scale histograms corresponding to the beverages prepared by mixing of fermented whey with fermented orange juice (W-OR) or fermented strawberry juice (W-ST)

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Table 3. Percentage of consumers who selected each of the terms of check-all-that-apply (CATA) question to describe each of the beverages prepared by mixing of fermented whey with fermented orange juice (W-OR) or fermented strawberry juice (W-ST)

		Samples	
Term	*P* values	W-OR	W-ST
Fruity***	0.0000	42	80
Orange Flavour^{ns}	0.7815	7	8
Refreshing^{ns}	0.8575	42	41
Murky^{ns}	0.4054	11	14
Thick^{ns}	1.0000	6	6
Sweet^{ns}	0.3359	37	32
Sour^{ns}	0.5050	35	31
Diluted**	0.0017	10	24
Bitter^{ns}	0.1317	9	4
Intense Colour***	0.0000	9	31
Concentrated*	0.0126	15	6
Natural^{ns}	0.5127	20	23
Alcohol**	0.0090	45	30
Acid^{ns}	0.4497	46	42
Artificial^{ns}	0.3458	19	15
Lactic Flavour***	0.0000	33	7
Smooth^{ns}	0.3359	32	37
Off Flavour^{ns}	0.1025	21	13
Astringent^{ns}	0.7815	12	13
Strawberry Flavour***	0.0000	1	49
Light Colour***	0.0000	38	12
Dry^{ns}	0.2253	21	16
Salty^{ns}	0.3173	5	3
Presence of Pulp***	0.0000	0	22

*** Indicates significant differences at $P < 0.001$.
** Indicates significant differences at $P < 0.01$.
* Indicates significant differences at $P < 0.05$.
^{ns}Indicates no significant differences ($P > 0.05$) according to Cochran's *Q* test.

1.4 g/l). Maximum theoretical yield for ethanol bioconversion from lactose fermentation is $Y_{max} = 0.538$ g ethanol/g lactose consumed (Ozmihci and Kargi, 2007). Ethanol yield values experimentally observed after 24 h were 0.54 ± 0.06, 0.43 ± 0.03, 0.34 ± 0.05, 0.46 ± 0.05 g/g for D, DW, HW and PER, respectively. High yield for ethanol production in the case of W medium may be explained in terms of better nutritional composition, as in the other media lower salt (DW) or protein (HW and PER) content were observed.

Ethanol yield found in this study as a consequence of *K. marxianus* growth on whey is similar to that found by Ozmihci and Kargi (2007) and higher than reported by others (Sansonetti *et al.*, 2009; Zoppellari and Bardi, 2013), suggesting good fermenting characteristics for *K. marxianus* LFIQK1. Higher ethanol content may be accomplished by increasing the initial substrate concentration, easily achievable for W, DW and HW media using higher whey powder concentrations in prepared solutions, as previously reported (Kosikowski and Wzorek, 1977; Yamahata *et al.*, 2020).

K. marxianus LFIQK1, showed interesting biotechnological characteristics in all media assayed, being able to grow and produce ethanol even in media that were partially reduced in nutrients, ie DW, HW and PER. For the next stage we chose whey matrix (W) as a lactose-rich medium for the later production of alcoholic beverages, because of the higher yield of ethanol observed. Nevertheless, the other whey-derived matrices assayed could also have been used.

Development of alcoholic beverages obtained by fermentation from whey and fruit juices

For both W-OR and W-ST beverages obtained by mixing fermented whey solutions (W) with fermented orange juice (OR) and strawberry pulp (ST) respectively, final pH values (Table 2) were similar to those corresponding to fruit juices before fermentation. Final SS values were higher for beverages than for individual fermented media, because of sucrose addition during preparation. Final alcoholic content of individual media and beverages are shown in Table 2. Final alcoholic content in OR and ST media were higher than for W media, in accordance with higher SS proportion observed for fruit media. Expressed in % (v/v), alcoholic level of W-OR and W-ST beverages (3.56 ± 0.03 and 2.1 ± 0.1% v/v, respectively) corresponded to relatively low alcohol graduation drinks. Different alternatives can be evaluated to obtain higher alcoholic graduation in beverages without ethanol addition. In the case of OR and ST matrices, this may be achieved with a longer fermentation time or by substrate (glucose or sucrose) addition before starting fermentation. In the case of the whey matrix, as discussed previously, reconstituted medium with higher whey powder concentration may be used.

The characteristics of colonies in cultured plates and cells under microscopic observation were similar to those typically observed for species belonging to *Saccharomyces* and *Kluyveromyces* genera, and no mould development was observed. Final counts were $2.5 \pm 0.2 \times 10^5$ CFU/ml for W-OR beverages and $1.7 \pm 0.4 \times 10^5$ CFU/ml for W-ST.

Sensory evaluation with consumers of the alcoholic beverages obtained by fermentation from whey and fruit juices

After W-OR and W-ST beverages evaluation by means of acceptability and CATA questions, significant differences were found in overall liking scores of the fermented beverages ($P < 0.05$), higher scores being found for W-ST (6.28 ± 1.46), than for W-OR beverage (5.77 ± 1.73). Good average acceptance (defined as a score above 5 on a 9-point hedonic scale) was observed for both beverages. Additionally, mean score for W-ST is similar to that reported previously for non-fermented lactic commercial beverages based on whey and strawberry pulp (Farah *et al.*, 2017). A histogram was constructed considering frequency of choice by consumers for each acceptability score in the 9-point hedonic scale (Fig. 1). The distributions were very similar for the two beverages. Additionally, most consumers scored 7 = like moderately (40.6% and 35.6% for W-ST and W-OR, respectively). Beverages were positively scored by 78 and 68 out of 101 consumers for W-ST and W-OR beverages, respectively. These observations suggest good sensory properties for the developed beverages, as scores upon indifference can be regarded as positive when

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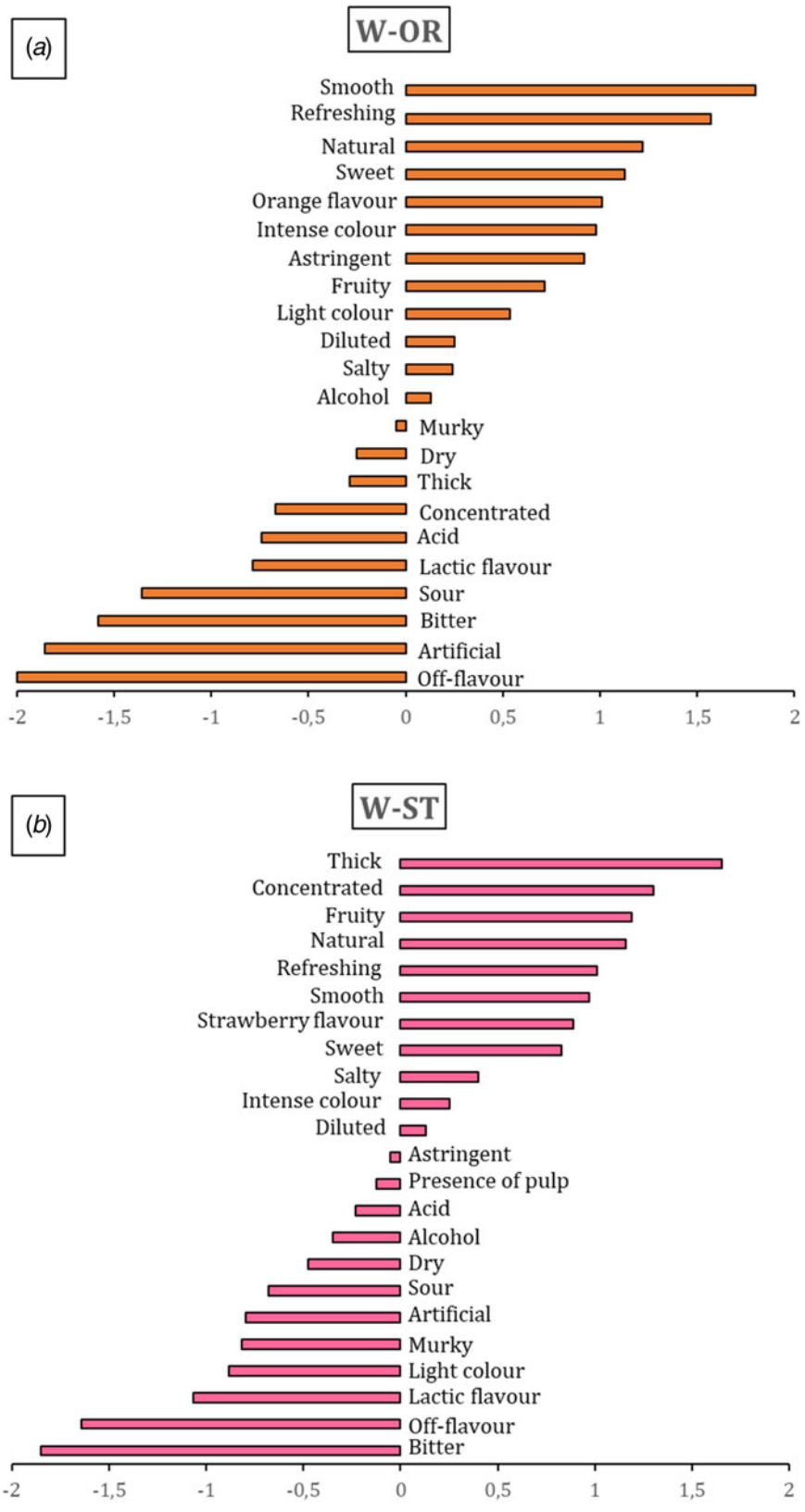

Figure 2. Penalty analysis for beverages prepared by (a) mixing of fermented whey with fermented orange juice (W-OR) or (b) fermented strawberry juice (W-ST).

evaluating new products for which consumers are not familiarized (Resende Oliveira *et al.*, 2018).

In Table 3, CATA terms are listed and the frequency consumers used each term for describing the beverages are reported. For each beverage, terms selected with higher frequency describe better (according to consumers) its sensory characteristics. Only considering those terms selected by at least 30% of consumers, W-OR beverage was described by the terms *fruity*, *refreshing*,

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sweet, *sour*, *alcohol*, *acid*, *lactic flavour*, *smooth* and *light colour*, while for W-ST beverage those terms were *fruity*, *refreshing*, *sweet*, *sour*, *intense colour*, *alcohol*, *acid*, *smooth* and *strawberry flavour*. For 9 of the 24 terms included in the CATA question, significant differences among samples were found in the frequency of use (Table 3). Six of them were marked with high frequencies for at least one of the beverages. This result suggests that the CATA question was able to detect differences in consumers' perception of the sensory characteristics in fermented beverages. Terms with significant differences among samples ($P < 0.05$) were: *fruity*, *diluted*, *intense colour*, *concentrated*, *alcohol*, *lactic flavour*, *strawberry flavour*, *light colour*, and *presence of pulp* (Table 3).

Penalty analysis was done in order to identify those descriptors driving acceptability. As shown in Fig. 2, for each beverage bars located on the right side of the graph correspond to descriptors positively contributing to product acceptability, and those on the left contribute negatively. The length of the bars indicates effect intensity. Descriptors present in both W-ST and W-OR beverages with higher positive influence on acceptability were *smooth*, *refreshing*, *natural*, *fruity* and *sweet*, while those with the most negative impact were *off-flavour*, *bitter*, *artificial*, *sour* and *lactic flavour*. More valuable information can be obtained when penalty analysis results are considered together with frequency of each attribute in the CATA question (Table 3). For W-OR beverage, attributes with higher contribution to acceptability according to penalty analysis were *smooth*, *refreshing*, *natural*, *sweet* and *orange flavour*. Among them, *smooth*, *refreshing* and *sweet* were selected by at least 30% of consumers, indicating their presence improves acceptance of beverages. The other two attributes with higher positive penalty values, *natural* and *orange flavour*, were scarcely checked by consumers, in agreement with high frequency observed for the attributes driving disliking *off-flavour* and *lactic flavour*. Sugar addition seems to be necessary in order to strengthen *sweet* and balance *sour* and *acid* attributes, and optimization of the level added must be explored. In general, consumers could not associate W-OR beverage to the fruit of origin. Low *orange flavour*, as well as high *off-flavour* and *lactic flavour* perception may be characteristics to take into account for beverage acceptability improvement.

The W-ST beverage was more clearly linked by consumers to the fruit of origin than W-OR. Attributes with higher positive penalty values (*fruity*, *refreshing*, *strawberry flavour* and *natural*) were frequently marked by participants, and attributes strongly associated to disliking (*bitter*, *off-flavour*, *lactic flavour* and *light colour*) were scarcely checked. In order to improve beverage quality, *thick* and *concentrated* attributes may be enhanced (e.g. by thickening and colouring agents' addition). For both beverages, acceptability may be improved by carbonation (Kappes *et al.*, 2007).

In conclusion, fermentative characteristics of the yeast *Kluyveromyces marxianus* LFIQK1 in different whey-derived matrices were explored in this work. In a 24-h fermentation assay, *K. marxianus* LFIQK1 cultured at 30 °C on whey (W) or whey-derived media (DW, HW and PER) without supplementation showed interesting characteristics related to lactose consumption and ethanol production. Very high ethanol yield was observed in W medium and high ethanol yield for DW and PER media, encouraging further research in utilization of these matrices. Two alcoholic beverages were developed by mixing equal parts of whey reconstitutes fermented with *K. marxianus* and fermented orange juice (W-OR) or juice from fermented strawberry pulp (W-ST), sweetened by sucrose addition. Sensory evaluation, based on acceptability and CATA questions, gave relevant information about the sensory characteristics of developed beverages. Comparatively, W-ST beverage showed higher acceptability values than W-OR beverage, but good acceptance from consumers was observed for both beverages, with mean acceptability values above indifference and more than two-thirds of participants expressing some degree of satisfaction. Penalty analysis allowed identification of descriptors with higher influence on acceptability, and suggests some improvements in order to increase beverage acceptability (addition of thickener agents to W-ST, enhancement of orange flavour in W-OR and carbonation or increasing sucrose level for both beverages). A novel alternative for utilization of whey and whey-related matrices by production of alcoholic beverages using natural ingredients can be proposed.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029924000116

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References

Ares G, Antúnez L, Bruzzone F, Vidal L, Giménez A, Pineau B, Beresford MK, Jin D, Paisley AG, Chheang SL, Roigard CM and Jaeger SR (2015) Comparison of sensory product profiles generated by trained assessors and consumers using CATA questions: four case studies with complex and/or similar samples. *Food Quality and Preference* **45**, 75–86.

Arshad UET, Hassan A, Ahmad T, Naeem M, Chaudhary MT, Abbas SQ, Randhawa MA, Pimentel TC, Gomez Da Cruz A and Aadil RM (2023) A recent glance on the valorisation of cheese whey for industrial prerogative: high-value-added products development and integrated reutilising strategies. *International Journal of Food Science and Technology* **58**, 2001–2013.

Barukčić I, Lisak Jakopović K and Božanić R (2019) Valorisation of whey and buttermilk for production of functional beverages – an overview of current possibilities. *Food Technology and Biotechnology* **57**, 448–460.

Comelli RN, Seluy LG and Isla MA (2016) Performance of several *Saccharomyces* strains for the alcoholic fermentation of sugar-sweetened high-strength wastewaters: comparative analysis and kinetic modelling. *New Biotechnology* **33**, 874–882.

Fagnani R, Puppio AAN and Zanon EO (2018) Sustainable alternative for the food industry: converting whey and orange juice into a micro-filtered beverage. *Scientia Agricola* **75**, 136–143.

Farah JS, Araujo CB and Melo L (2017) Analysis of yoghurts', whey-based beverages' and fermented milks' labels and differences on their sensory profiles and acceptance. *International Dairy Journal* **68**, 17–22.

Fonseca GG, Heinzle E, Wittmann C and Gombert AK (2008) The yeast *Kluyveromyces marxianus* and its biotechnological potential. *Applied Microbiology and Biotechnology* **79**, 339–354.

Guimarães PM, Teixeira JA and Domingues L (2010) Fermentation of lactose to bio-ethanol by yeasts as part of integrated solutions for the valorisation of cheese whey. *Biotechnology Advances* **28**, 375–384.

Janiaski DR, Pimentel TC, Cruz AG and Prudencio SH (2016) Strawberry-flavored yogurts and whey beverages: what is the sensory profile of the ideal product? *Journal of Dairy Science* **99**, 5273–5283.

Kadyan S, Rashmi HM, Pradhan D, Kumari A, Chaudhari A and Deshwal GK (2021) Effect of lactic acid bacteria and yeast fermentation on antimicrobial, antioxidative and metabolomic profile of naturally carbonated probiotic whey drink. *LWT* **142**, 111059.

Kappes SM, Schmidt SJ and Lee SY (2007) Relationship between physical properties and sensory attributes of carbonated beverages. *Journal of Food Science* **72**, S001–S011.

Kosikowski FV and Wzorek W (1977) Whey wine from concentrates of reconstituted acid whey powder. *Journal of Dairy Science* **60**, 1982–1986.

Lane MM and Morrissey JP (2010) *Kluyveromyces marxianus*: a yeast emerging from its sister's shadow. *Fungal Biology Reviews* **24**, 17–26.

Manoukian EB (1986) *Mathematical Nonparametric Statistics*. New York, NY: Gordon and Breach.

Ozmihci S and Kargi F (2007) Kinetics of batch ethanol fermentation of cheese-whey powder (CWP) solution as function of substrate and yeast concentrations. *Bioresource Technology* **98**, 2978–2984.

Panesar PS and Kennedy JF (2012) Biotechnological approaches for the value addition of whey. *Critical Reviews in Biotechnology* **32**, 327–348.

Pescuma M, Hébert EM, Mozzi F and De Valdez GF (2010) Functional fermented whey-based beverage using lactic acid bacteria. *International Journal of Food Microbiology* **141**, 73–81.

Resende Oliveira ER, Caliari M, Júnior MS, Ribeiro Oliveira A, Marques Duarte RC and Barros Vilas Boas EVD (2018) Assessment of chemical and sensory quality of sugarcane alcoholic fermented beverage. *Journal of Food Science and Technology* **55**, 72–81.

Sansonetti S, Curcio S, Calabrò V and Iorio G (2009) Bio-ethanol production by fermentation of ricotta cheese whey as an effective alternative non-vegetable source. *Biomass and Bioenergy* **33**, 1687–1692.

Varela P and Ares G (2014) *Novel Techniques in Sensory Characterization and Consumer Profiling*. Boca Ratón, FL, USA: CRC Press.

Yamahata N, Toyotake Y, Kunieda S and Wakayama M (2020) Application of multiple sensory evaluations to produce fermented beverages made from sole whey using *Kluyveromyces marxianus*. *International Journal of Food Science and Technology* **55**, 1698–1704.

Zafar S and Owais M (2006) Ethanol production from crude whey by *Kluyveromyces marxianus*. *Biochemical Engineering Journal* **27**, 295–298.

Zoppellari F and Bardi L (2013) Production of bioethanol from effluents of the dairy industry by *Kluyveromyces marxianus*. *New Biotechnology* **30**, 607–613.

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