

Functional fermented whey carrot beverage - qualitative, nutritive and techno-economic analysis

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Abstract

The main objectives of the paper were to investigate the possibility of use of carrot juice in the production of functional fermented whey based beverage, to simulate and economically evaluate the production process and the possibility for its inclusion in the process of cheese production. Quality of produced whey carrot beverage was evaluated through the estimation of pH value, titratable acidity, syneresis, viscosity, sensory value, antioxidant activity and viable cell count of probiotic bacteria. The production process was simulated and economically evaluated using SuperPro Designer 5.1 software. Based on the results, the integrated cheese/whey carrot beverage production process showed greater economic viability compared to the basic cheese production process. Integrated process of cheese/whey carrot beverage production allows a fast return of capital (0.15 years), high net present value - NPV (10464.04) and internal rate of return - IRR (384.61) values. Obtained whey carrot beverage with following quality parameters: antioxidant activity 90.5%, viable cell count 8.7 log (CFU/mL), pH value 4.6, titratable acidity 20.0 °SH, syneresis 66.7%, viscosity 2.693 cP and sensory value 8.97 represents the beverage with great potential in consumer acceptance. Integration of beverage production line into the cheese production process, delivers the product with improved quality, which can meet the sophisticated consumer's demands and can increase the overall profit of the dairy industry.

Keywords: carrot beverage, SuperPro Designer, process simulation, techno-economic analysis, integrated cheese/whey beverage production.

1. Introduction

Whey is the portion of milk that remains after the coagulation of casein, during the process of cheese production and contains about half of the milk solids. Due to the high biological oxygen demand (BOD) (39,000 to 48,000 ppm), development of processes of its economical utilization would be the great benefit to the dairy industry. In that sense, bioavailability of whey should be exploited by diversification of whey based products through the economically profitable processes (DUBEY & al. [1]).

Market demand for healthy beverages is growing all over the world. Over the past few decades, probiotic beverages are usually marketed in the form of fermented milks and yoghurts. In recent years in line with the increasing consumer demand for the health benefits products, researchers begin to develop new probiotic products based on whey (VASUDHA & al. [2]). Numerous approaches have been taken in an effort to transform a large volume of

whey into valuable products suitable for use as food (GIRSH & al. [3], REUST & al. [4]). Whey is a rich source of high-quality proteins, minerals, vitamins and lactose that can be utilized and transformed in numerous valuable products (lactose, protein concentrates, lactalbumin, lactoglobulin, galactose, glucose, etc.) through various processes such as concentration and/or fractionation, drying, fermentation or hydrolysis (CARIĆ & al. [5]). However, given that all these processes are quite expensive, require advanced technologies and do not lead to full exploitation of raw material, the most economical way of whey processing is the production of functional fermented whey based beverages.

As whey has an unappealing taste, weak texture, poor mouthfeel, relatively high lactose-to-glucose ratio and excessive acidity, especially if it belongs to the class of acid whey, numerous procedures have been developed for improving its characteristics, aiming to enable its more comfortable utilization in human nutrition (DJURIĆ & al. [6]). Application of probiotic cultures, or its combination with specially designed dairy cultures, could significantly improve quality of whey based beverages (BULATOVIĆ & al. [7]). Likewise, the inclusion of vegetable or fruit juices into beverages leads to the additional development of formulations and the creation of products that meet sensitive consumers demands related to the pleasant taste of products. Masking is one technique that has been used to reduce the sensations of unpleasant odors and flavors in foods and it has been performed successfully through the addition of new substances with acceptable flavors (REINECCIUS & al. [8]), and is therefore supposed to be capable of reducing the negative sensory attributes. The addition of fruit and vegetable juices might positively contribute to the aroma and flavor of the final product and might avoid the identification of off-flavors by consumers (LUCKOW & al. [9]).

In nutritional manner, whey have been recognized as an effective thirst quencher, light, refreshing, healthful and nutritious beverage (PRENDERGAST & al. [10]). Whey and its biological components have positive effects in treatments of several chronic diseases like cancer, cardiovascular, HIV etc., and are more suitable for health as compared to other drinks (SARVANA KUMAR & al. [11]). In addition to the role in fermentation processes, probiotic lactic acid bacteria are dietary sources of live microorganisms destined to promote a positive impact in the host by improving the properties of the indigenous beneficial microbiota (KLAENHAMMER & al. [12]). On the other hand, probiotic cultures are known to improve the lactose digestion in lactose maldigestors (GILLILAND & al. [13]). Enrichment with vegetable and fruit juices, introduces a new value of these products by incorporation of valuable substances, like bioactive peptides, amino acids, oligosaccharides that exert different biological activities.

There are a small number of papers that investigate the possibility of using the carrot juice in the formulation of whey based beverage. In addition, there is no paper that a comprehensively analyzes the production process of this type of beverage in qualitative, nutritive and technological sense. The aim of this paper was to investigate the possibility of use of carrot juice in the production of functional fermented whey based beverage. In addition to the qualitative examination, the study also reported simulation of the production process and the possibility for its inclusion in the process of cheese production, together with the simultaneous economic analysis.

2. Materials and Methods

2.1. Culture and media

Commercial lyophilized dairy starter culture 'Lactoferm ABY 6' used in this study was supplied by Biochem s.r.l. (Monterotondo, Roma, Italy). Starter culture is a mixture of yogurt (*Streptococcus salivarius* ssp. *thermophilus* (80%), *Lactobacillus delbrueckii* ssp. *bulgaricus* (1%)) and probiotic (*Lactobacillus acidophilus* (13%), *Bifidobacterium bifidum* (6%)) bacteria. The culture that consists of 10 g lyophilised starter powder is the one currently used in dairy industry. The culture was maintained according to the manufacturer's instructions at -

18 °C until use (no longer than 20 months). For each experiment, 1% (w/v) of starter culture was gently dissolved in sterilized skim milk (0.5% fat) and activated 30 min at 42 °C.

Whey remained after cheese production and sterile skim milk with 0.5% fat were obtained from dairy plant Imlek a.d. (Belgrade, Serbia). After collection, the whey was stored at -18 °C until use (no longer than one week). The chemical composition of whey was: proteins 2.6 ± 0.012 % (w/v); fats 1.05 ± 0.08 % (w/v) and lactose 5.6 ± 0.114 % (w/v). The chemical composition of milk was: proteins 3.1 ± 0.012 % (w/v); fats 0.5 ± 0.08 % (w/v) and lactose 4.7 ± 0.114 % (w/v).

Carrot juice (Foodland doo, Beograd, Serbia) used in the experiments is commercial pasteurized juice. The chemical composition of carrot juice was: carbohydrates 13.27 % (w/v); fats 0.03 % (w/v), proteins 0.12 (w/v).

2.2. Experimental procedure

The flasks containing 300 mL of formulated samples with following compositions: whey-milk beverage - WMB (70% whey, 30% milk, v/v) and whey-carrot beverage - WCB (40% whey, 30% milk, 30% carrot juice, v/v), were prepared for each point of analyses. Mixtures were pasteurized at 60 °C for 60 min, cooled at fermentation temperature (42 °C) and inoculated with 6% (v/v) of activated culture. Prior to pasteurization, pH value of formulated whey-carrot beverage mixtures was set on 6.5 by 2.0% (w/v) aqueous solution of sodium hydrogen carbonate (NaHCO₃, Sigma-Aldrich, Australia). Formulated samples were incubated at 42 °C in a water bath. During the incubation, samples (2 mL) were taken every 1h for determination of pH value. The fermentations were carried out for 4h until pH = 4.60 ± 0.20 was attained. After 4h, fermentations were stopped by quick cooling. The fermented beverages were stored at 4 °C for 28 days. Analysis of the titratable acidity (TA, °SH), pH value, viable cell count (log (CFU mL⁻¹)), syneresis (%), viscosity (cP), antioxidant activity (%) and sensory value was carried out before fermentation and after the 0, 14 and 28 days of storage. After the suggesting the manufacturing process, the design and cost-benefit analysis of the production process was carried out using the SuperPro Designer 5.1. Software (Intelligen, Inc.).

2.3. Chemical analysis

The titratable acidity was determined by the Soxhlet-Henkel method (VARGA & al. [14]), and the pH value was measured using a pH meter (Inolab, WTW 82362, Wellheim, Germany).

2.4. Microbiological analysis

One milliliter of fermented sample was diluted with 9 mL of saline (0.85%, w/v), and mixed uniformly. Subsequent serial dilutions were prepared and viable cell count was determined using pour plate technique. The viable cell count was estimated using the pour plate technique on MRS-maltose (MRSM) agar as selective media for enumeration of probiotic bacteria *L. acidophilus* and *B. bifidum*, after 48 h of incubation in anaerobic jars at 37 °C. (DAVE & al. [15]).

2.5. Texture analysis

2.5.1. Viscosity

The apparent viscosity of tested samples was determined at 8 °C according to the modified method (ARYANA & al. [16]). A Brookfield DV II+ Pro viscometer (Brookfield Engineering Lab Inc., Stoughton, MA) was used. A spindle N°61 was set to 10 rpm. The viscosity measurements were continuous over 30 s required to collect 70 data points. Data points were averaged per sample per replication. The apparent viscosity was determined on three cups of sample per replication. Three replications were conducted and values are expressed in cP.

2.5.2. Syneresis

Syneresis of fermented samples was determined according to the method (KEOGH & al. [17]). The fermented samples (20.0 mL) were centrifuged at 1000 rpm for 10 min at 4 ± 1 °C. Collected supernatant was drained, weighed and the following equation was used for syneresis calculation:

$$\text{Syneresis(\%)} = \frac{\text{Weight of supernatant (g)}}{\text{Weight of fermented sample(g)}} \times 100\% \quad (1)$$

2.6. Sensory analysis

Sensory analysis of fermented samples was conducted after 0, 14 and 28 days of storage according to the modified method (HEMSWORTH & al. [18]). Fifty-five untrained panellists (35 being women and 20 men, age between 25 and 55) from the faculty, including teachers, students and staff were randomly selected and invited to participate in the sensory evaluation of fermented whey-based beverages on the basis overall acceptability. The participants were asked to assess the overall acceptability of the two different fermented beverages: WMB and WCB. Each questionnaire consists of four questions: name, age, sex and overall acceptability for two consumed products.

The samples were presented monadically at 4 ± 1 °C, in individual plastic cups coded with 3-digit numbers, serving 20 mL samples to each panellist. The participants were given two samples at a time, at storage temperature (4 ± 1 °C), a pencil, a questionnaire and a glass of cold water to rinse their mouths between samples. They have been asked to mark a value on the questionnaire scale which best represents how much they liked or disliked each of four samples with respect to overall acceptance, using a 9-point hybrid hedonic scale where 1 = disliked extremely; 5 = neither liked nor disliked and 9 = liked extremely. The sensory analysis was consisted of 110 questionnaires distributed into 4 sessions (4 times of storage). Prior to serving all samples were subjected to counts of yeasts, molds and coliforms using standard microbiological methods to evaluate the hygienic and sanitary conditions of the products.

2.7. Antioxidant activity analysis

Antioxidant activity of fermented whey-based beverages was determined by its ability to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, which was measured according to the modified method (BALAKRISHNAN & al. [19]). A stock solution of 0.1 mM DPPH (Sigma-Aldrich, Australia) was prepared by dissolving in methanol. After 4h fermentation samples were macerated with methanol and centrifuged at 8000 rpm for 20 min at 4 °C. Methanol (1.5 mL) and DPPH (1.0 mL) were added to the supernatant (0.5 mL). Control sample was prepared by mixing methanol (1.5 mL) and DPPH (1.5 mL), while methanol was used as blank sample. Mixtures were allowed to stand 30 min in the dark, at room temperature. The antioxidant activity was analyzed by reading the absorbance at 517 nm. Scavenging activity was calculated using the following equation (UNAL & al. [20]):

$$\text{DPPH scavenging activity (\%)} = \left[\frac{A_c - A_a}{A_c} \right] \times 100 \quad (2)$$

Where A_a and A_c represent absorbance of sample and control, respectively.

2.8. Statistical analysis

The experiments were performed in triplicate. All values are expressed as mean \pm standard deviation. Mean values were analyzed using two-way ANOVA. The Tukey post hoc test was performed for means comparison (Origin Pro 8 (1991-2007), Origin Lab Co., Northampton, USA). Differences were considered significant at $P < 0.05$.

3. Results and Conclusions

3.1. Quality approach

Chemical and sensory analysis

Titrate acidity and pH value are the main chemical parameters that have impact on acceptability of fermented beverages, which determine does the beverage satisfy necessary criteria for a particular product group. Lactic acid is main component that has significant impact

on the flavor of fermented milk products. Based on the literature (PINTHONG & al. [21], KEHAGIAS & al. [22]), titratable acidity above 53 °SH is marked as acidity that causes unpleasant and unacceptable acid taste of whey based products. As shown in Table 1, after 4h of fermentation (0 day) pH value of 4.40 ± 0.20 was reached in both samples. As can be seen, sample with carrot juice (WCB) has higher pH value (4.60 ± 0.06), after the fermentation as well as during whole storage period, then sample WMB (4.40 ± 0.04) formulated without carrot juice addition. Observed results are in agreement with lower titratable acidity observed in sample WCB (20.0 ± 0.1 °SH) compared to the sample WMB (23.2 ± 0.2 °SH) after the fermentation, as well as during whole storage period. This could be explained by different fermentative activity of ABY-6 culture in substrate that's abundant with different type of carbohydrates and proteins. Based on this observation, it could be assumed that metabolic activity of ABY-6 culture is probably directed to the production of other valuable metabolites rather than lactic acid. However, gradually decrease of pH value and increase of titratable acidity observed in all samples during the storage period does not reach the extreme values of parameters.

Comparing the quality parameters after the fermentation (0 day) it can be seen that the WCB sample has lower syneresis and viscosity ($64.7 \pm 0.40\%$, 2.693 ± 0.014 cP respectively) compared to the beverage WMB ($67.5 \pm 0.70\%$, $2,702 \pm 0,012$ respectively). Presented results suggest that supplementation with carrot juice affects viscosity and syneresis of produced beverage, but do not have dramatically negative impact on the structure of beverage i.e. the formation of protein matrix that is crucial to the beverage texture. The same trend of differences in the quality parameters values, in favour to the beverage WCB, was recorded throughout the storage period. Thus, during the whole storage period, the beverage WCB exhibits and maintains superior quality compared to the beverage WMB.

In addition, due to the synergy effect of pleasant taste of carrot juice and lower acidity, sample WCB achieved higher sensory values (Table 1) in each point of analysis. It could be said that carrot juice contributes to the better sensorial sensation of produced beverage. Maximal sensory values were obtained for sample WCB ($8,97 \pm 1,21$) after the fermentation and after 14 days of storage ($8,51 \pm 1,32$).

Comparing the quality parameters of the beverage WCB (40% whey, milk 30.0% and 30.0% carrot juice) and beverage WMB (70% whey and milk 30.0%) it can be concluded that the addition of 30.0% carrot juice improves sensory attributes of the functional fermented whey based beverage.

Table 1. Quality parameters of whey-milk (WMB) and whey-carrot (WCB) beverage

Quality parameter	Sample	Before fermentation	Time of storage, day		
			0	14	28
pH value	WMB	$6,50 \pm 0,01$	$4,40 \pm 0,04$	$4,10 \pm 0,01$	$3,88 \pm 0,03$
	WCB	$6,50 \pm 0,02$	$4,60 \pm 0,06$	$4,49 \pm 0,03$	$4,47 \pm 0,05$
Titratable acidity (°SH)	WMB	$4,43 \pm 0,06$	$23,2 \pm 0,20$	$30,6 \pm 0,30$	$31,2 \pm 0,20$
	WCB	$4,80 \pm 0,04$	$20,0 \pm 0,10$	$22,6 \pm 0,50$	$25,2 \pm 0,10$
Syneresis (%)	WMB	$100,0 \pm 0,00$	$67,5 \pm 0,70$	$75,1 \pm 0,75$	$78,3 \pm 0,76$
	WCB	$90,0 \pm 0,00$	$66,7 \pm 0,70$	$72,1 \pm 0,72$	$75,3 \pm 0,68$
Viscosity (cP)	WMB	$1,353 \pm 0,022$	$2,702 \pm 0,012$	$2,835 \pm 0,023$	$2,953 \pm 0,026$
	WCB	$1,553 \pm 0,011$	$2,693 \pm 0,014$	$2,793 \pm 0,042$	$2,981 \pm 0,019$
Sensory value	WMB	$6,51 \pm 1,01$	$8,52 \pm 1,01$	$8,40 \pm 1,15$	$8,12 \pm 1,13$
	WCB	$7,41 \pm 1,45$	$8,97 \pm 1,21$	$8,51 \pm 1,32$	$8,28 \pm 1,41$

3.2. Nutritive approach

Microbiological and Antioxidant activity analysis

In nutritive manner, functional fermented whey based beverage can be characterized based on the level of functional constituents that contributes to its nutritive quality.

Based on the results related to the nutritive quality parameters registered after the fermentation (0 day), it can be seen that antioxidant activity ($91.3 \pm 0.06\%$) of WCB beverage was significantly higher compared to the beverage WMB ($46.0 \pm 0.07\%$) (Table 2). Obtained results are in agreement with literature, and could be explained by high antioxidant capacity (60.0%) of nutrients present in carrot juice, that also leads to the higher proteolytic activity of ABY-6 culture (KRUPA & al. [23], CAGNOA & al. [24], MADHU & al. [25]). Exactly the same trend of differences in the antioxidant activity values, in favour to the beverage WCB, was recorded throughout the storage period. Sufficient viable cell count (in range 8.65-8.80 log (CFU/mL)) was reached in both samples after the fermentation as well as during the whole storage period. Achieved viable cell count was higher than count reported in previous studies (BULATOVIĆ & al. [7], BULATOVIĆ & al. [26]), related to the growth of bacteria in the whey, which could be explained by abundance of nutrients in formulated substrate that are originated from carrot juice. Thus, during all of the 28 days of storage, the beverage WCB exhibits and maintains superior antioxidative quality compared to the beverage WMB.

From the above findings, it can be concluded that the addition of 30.0% of carrot juice in a formulation comprising 40.0% whey and 30% milk leads to a significant improvement of the nutritive quality of the beverage. The produced beverage has the following composition: carbohydrates 5.7%, fats 0.58%, proteins 2.0%.

Table 2. Nutritive quality parameters of whey-milk (WMB) and whey-carrot (WCB) beverage

Quality parameter	Sample	Before fermentation	Time of storage, day			
			0	1	4	28
Antioxidant activity (%)	WMB	$15,0 \pm 0,07$	$46,0 \pm 0,07$	$38,1 \pm 1,09$	$39,8 \pm 0,08$	
	WCB	$76,1 \pm 0,05$	$90,5 \pm 0,09$	$87,2 \pm 1,03$	$94,6 \pm 0,07$	
Viable cell count (log (CFU/mL))	WMB	$6,61 \pm 0,123$	$8,65 \pm 0,132$	$8,46 \pm 0,143$	$8,19 \pm 0,116$	
	WCB	$6,41 \pm 0,154$	$8,66 \pm 0,182$	$8,80 \pm 0,187$	$8,20 \pm 0,134$	

3.3 Technological approach

Simulation - Whey Carrot Beverage Production

The composition of whey, milk and carrot juice considered in the computational simulation was described in Section 2.1. The product obtained through present simulation has direct application in human nutrition, and the entire process has no revenue streams with environmental impact. Main characteristics of the unit operations involved in the scenario proposed for the WCB making section are shown in Table 3, while simplified flowcharts of WCB and integrated Cheese-WCB production are shown in Figure 1.

Table 3. Main characteristics of the unit operations involved in the scenario proposed for the WCB making section.

Unit ID	Description	Detail
V-101	Blending/Storage Tank	Exit temperature 4°C
HX-101	Heating/Pasteurization	Exit temperature 60 °C
HX-102	Cooling	Residence time 1h
V-102	Fermentation	Exit temperature 42 °C
HG-101	Homogenization	Residence time 2.5h
HX-103	Cooling	Exit temperature 42°C
FL-101	Filling	Residence time 1h
		Exit temperature 10 °C
		25 kW Operating power

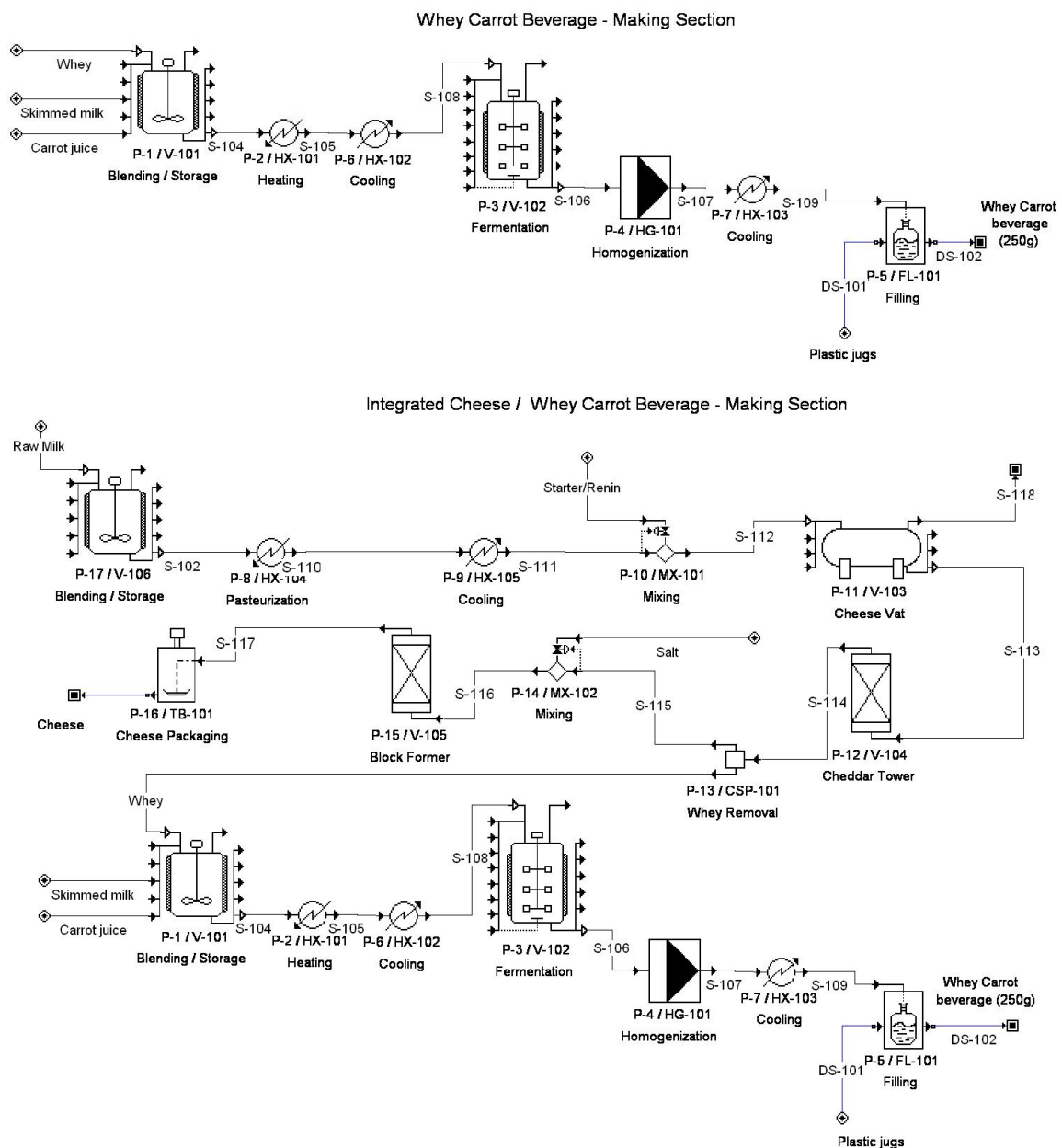


Figure 1. Simplified flowcharts of Whey Carrot Beverage and Integrated Cheese / Whey Carrot Beverage production lines

Economical analysis

In the financial evaluation of the WCB production scenario following economic indicators were analyzed: Payback Period (PP), Net Present Value (NPV) and Internal Rate of Return (IRR). Parameters permit comparison of projects with different investment dimensions, and were used for comparison of processes with included variations in sales prices of the final products and the purchase price of raw materials. Summary of the economic parameters of the plants for the analyzed models is shown in Table 4. For the purpose of analysis and evaluation of economic viability of integration of whey carrot beverage production process into the cheese production process, the economic indicators were compared with indicators for cheese production process scenario (taken from SuperPro Designer 5.1 - Example files).

Table 4. Summary of the economic parameters of the plants for the models analyzed.

Parameter	Cheese	Whey carrot beverage	Integrated Cheese - Whey carrot beverage
Total investment (\$ · 10 ⁶)	32.570	157.620	210.508
Payback Period - PP (year)	1.67	0.18	0.15
Net Present Value - NPV (\$ · 10 ⁶)	112.218	6463.88	10464.04
Internal Rate of Return - IRR (%)	53.36	312.89	384.61

The results presented in Table 4, that are related to the economic impact of completely new plants, allow verifying that all processes are economically viable due to the low PP, as well as high NPV and IRR.

The simulation of plant used for production of WCB is noticeably more attractive when compared to the simulation of plant for cheese production, and there is greater viability compared to cheese production plant. This is due to the tendency of the economic indicators, since it allows a faster return of capital (0.18 years) with higher NPV and IRR values compared to cheese production process indicators (Table 4). Designed processes are more attractive than those reported in the literature (DA SILVA & al. [27]) related to the integrated whey protein concentrate (WPC) production, due to the shorter payback period. In addition, integration of WCB production process into the cheese production process, allows significant decrease in PP value (0.15 years) as well as increase of NPV (10464.04) and IRR (384.61) values compared to the individual cases of cheese and WCB production processes.

Given the fact that the WCB production process is practically the same as the processes engaged in yogurt production, which already exist in dairy plant facilities, its integration practically requires negligible investments and lead to the direct economic benefit.

The integrated cheese/whey carrot beverage production process showed greater economic viability than basic cheese production process. Due to the excellent economic indicators, since the integrated process of cheese / whey carrot beverage production allows a faster return of capital (PP), with higher NPV and IRR, plant that simultaneously produces cheese and whey carrot beverage is more economically attractive when compared to the plant that produces only a cheese. Obtained whey carrot beverage with following quality parameters: antioxidant activity 90.5%, viable cell count 8.66 log (CFU/mL), pH value 4.60, titratable acidity 20.0 °SH, syneresis 66.7%, viscosity 2.693 cP and sensory value 8.97 represents the beverage with great potential in consumer acceptance. Integration of beverage production line into the cheese production process, delivers the product with improved quality, which can meet the sophisticated consumer's demands and can increase the overall profit of dairy industry.

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