



# Bioconversion of potato waste by rumen fluid from slaughterhouses to produce a potential feed additive rich in volatile fatty acids for farm animals



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## ABSTRACT

Potato waste, usually derived from storage and processing of potatoes, is an agricultural byproduct already used as a feed for ruminants. Its fermentation using rumen fluid, another waste product obtainable from slaughterhouses, is expected to valorize both products. Bioconversion of potato waste using rumen fluid was followed by measuring volatile fatty acids (VFAs), pH, and the extent of digestion of the potato waste as well as the nutrient composition of the solid residues after fermentation. Different ratios (1:2, 2:2, 3:2 and 4:2 w dry matter/v) of potato waste to rumen fluid were fermented in a Daisy<sup>II</sup> incubator for 12 or 24 h at 39 °C. Depending on the fermentation time and the dry mass of potato waste used for fermentation, a digestion rate between 31 and 78.8% of the potato waste (solid phase of the fermentation mixture) was obtained. Fermentation resulted also in an up to 2.5-fold increase in the concentration of VFAs in the liquid phase of the fermentation mixture. During fermentation, the pH value of the fermentation mixture dropped from 6.95 to about 4.0 ( $P < 0.05$ ). The highest digestion rate and the highest VFA production were obtained with 100 g potato waste and a 24 h fermentation time. Furthermore, the residual solid residues after fermentation had higher protein and fat contents compared to the potato waste used for fermentation ( $P < 0.05$ ). Based on the results obtained, potential applications of both potato wastes and rumen fluids have been identified. The used method for bioconversion is suggested for scale-up purposes.

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## 1. Introduction

One of the major environmental problems of the world today is caused by a continuous increase in the production of organic wastes, especially agricultural wastes. Therefore, sustainable management including the reduction of organic wastes became a main priority in many countries in order to prevent environmental pollution (Khalid et al., 2011). Potato (*Solanum tuberosum*) represents one of the most important crops in many countries and over

the past 20 years, its global production steadily increased to 365 Mt (FAO, 2015). However, 35% of the potatoes harvested worldwide were estimated to be discarded during storage and processing (Tawila et al., 2008). Therefore, a suitable utilization should be considered to overcome the environmental problems linked to potato waste. Management strategies for potato waste include its application as a fertilizer for cropland or as a feed for ruminants (Nelson, 2010). Since potato waste consists mainly of carbohydrates, and to some extent of proteins, and lipids, it represents an excellent source for fermentative bioconversion to generate products with higher economic and nutritive values such as probiotics, prebiotics, organic acids and microbial proteins. Fermentation of carbohydrate-containing agricultural waste using a mixture of microorganisms was already shown to be a promising approach to convert the carbohydrates into microbial protein (Ghanem, 1992). Among others, anaerobic digestion has found increasing attention

**Abbreviations:** VFAs, volatile fatty acids; DM, dry matter; DOM, digestible organic matter; ME, metabolizable energy).

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for bioconversion of organic wastes. Thereby, complex insoluble macromolecules are hydrolyzed in a multistage process by different microorganisms in a concerted action into simpler and more soluble compounds (Sonakya et al., 2001). In this regard, rumen microorganisms could be considered to be effective in bioconversion of agricultural wastes, because the rumen displays an anaerobic microbial ecosystem with cellulolytic and amylolytic activities (Yue et al., 2013). Rumen fluid is a waste product from slaughterhouses and accumulates from ruminants slaughtered daily worldwide. Because of its release into the environment, problems of odor, flies and hygiene, pollution of surface and ground waters with pathogens and undesirable chemical compounds are occurring. Therefore, bringing rumen fluid to useful applications will solve an additional environmental problem (Rezai-Sarteshnizi et al., 2018).

Research works have already been focused on the application of rumen microorganisms for anaerobic bioconversion of lignocellulosic biomass (Barnes and Keller, 2004; Yue and Yu, 2009). However, ruminal cellulolytic bacteria are more sensitive than ruminal amylolytic bacteria to the pH of the growth medium and they were shown to be unable to digest cellulose at low pH (Russell and Wilson, 1996). Therefore, pH control is the main problem when using the lignocellulosic biomasses as the substrate for bioconversion by rumen cultures (Yue et al., 2007). By using amylolytic biomasses, such as potato waste, a pH control during fermentation is however not needed. Fermentation of potato wastes by rumen microbes is expected to result in microbial growth and production of volatile fatty acids (VFA) including acetic, propionic, butyric, valeric and iso-valeric acids.

VFAs were reported to be the main products of anaerobic digestion of agricultural wastes with a wide range of application in food and pharmaceutical industries and in the production of esters, bioplastics and bioenergy (Fang et al., 2020). In addition, VFAs are extensively used in animal nutrition as feed additives to support animal health. It has been established that these acids are effective in reducing pathogen colonization in the intestine. This results in improved animal growth, better feed efficiency and therefore in an improve health status of the animals (Khan and Iqbal, 2016). To the best of our knowledge, there was no study performed focusing on bioconversion of amylolytic biomasses such as potato waste by rumen microorganisms to produce VFAs. Furthermore, fermentation of potato waste by rumen microorganisms was expected to result in a product high in proteins because rumen microorganisms have been shown to be capable of converting carbohydrates to proteins (Aziz and Mohsen, 2002). Such a process will help to reduce the environmental problems cause by both potato waste and rumen fluids.

## 2. Materials and methods

### 2.1. Preparation of the potato waste for fermentation

Potato waste (cracked potatoes, damaged tubers obtained from harvesting, deformed and glassy tuber, under-sized tubers, tubers injured by insects and pests) were collected from local depots in Ardabil province, Iran. Dust was removed by washing with water. The potatoes were cooked for 20 min in water to prevent the negative effect on rumen microbial activity triggered by potato waste exposed to a rapid enzymatic browning and oxidation. Thereafter, the cooked potatoes were ground to obtain a uniform substrate for fermentation.

### 2.2. Fermentation of the potato waste by rumen microorganisms

Rumen contents were collected from the rumen content pool of a slaughterhouse (Ardabil industrial meat complex, Ardabil, Iran).

After transportation of the rumen contents to the laboratory using pre-warmed containers, they were homogenized under constant CO<sub>2</sub> purging using a laboratory blender. This mixture was filtered through four-layer cheesecloth to remove any solids. Fermentation was performed with different ratios (1:2, 2:2, 3:2 and 4:2 w dry matter/v) of potato waste to rumen fluid. 100 g (P1R2), 200 g (P2R2), 300 g (P3R2) and 400 g (P4R2) of potato waste were fermented in the presence of 200 mL rumen fluid in a Daisy<sup>II</sup> incubator (capacity 2 L) for 12 or 24 h at 39 °C. After adding ground potato waste and rumen fluid to the fermentation flasks, the content and the headspace of the flasks were flushed with CO<sub>2</sub> thoroughly. Thereafter, the fermentation flasks were closed tightly and fermentation was performed under slow rotation (2 cycles per minute). After fermentation, the fermentation mixtures were passed through one-layer cheesecloth and the solid and liquid phases were collected separately for further analysis. The reduction in the dry mass of the potato waste (solid phase of the fermentation mixture) by fermentation was considered as digested and expressed as the percentage of the dry mass of the potato waste before fermentation.

### 2.3. Analytical methods

Potato waste samples (before and after fermentation) were dried for 24 h at 105 °C. Prior to be used for compositional analysis, this material was ground with a laboratory grinder in order to pass a 1 mm sieve. AOAC methods (1990) were used to determine crude ash, ether extract, crude protein and DM (dry matter). A factor of 6.25 was used to calculate the crude protein. The ANKOM Technology Method (Van Soest et al., 1991) was applied to quantify neutral detergent fiber. The content of non-fibrous carbohydrates was calculated by subtraction of the sum of the percentages of the ether extracts, neutral detergent fiber, crude protein and ash from 100 (NRC, 2001). DOM (digestible organic matter) and ME (metabolizable energy) were calculated by equations (1) and (2), respectively (Menke and Staingass, 1988):

$$\text{DOM (\% of DM)} = (0.9991 \times \text{GP}) + (0.0595 \times \text{CP}) + (0.0181 \times \text{CA}) + 9 \quad [1]$$

$$\text{ME (MJ/kg DM}^{-1}\text{)} = 1.06 + (0.157 \times \text{GP}) + (0.0084 \times \text{CP}) + (0.022 \times \text{EE}) - (0.0081 \times \text{CA}) \quad [2]$$

GP represents gas production (mL) from 200 mg dry matter potato waste within 24 h. CA, CP, and EE represent crude ash, crude protein, and ether extract expressed as the percentage of DM, respectively.

Immediately after collecting the rumen fluid after fermentation (liquid phase of the fermentation mixture), its pH was recorded with a laboratory pH meter (Metrohm pH-meter). Aliquot of the rumen fluid samples were stabilized with a few drops of 50% (v/v) sulfuric acid and kept at -20 °C until quantification of VFAs.

Prior to VFAs quantification, thawing of the samples was performed at 4 °C. Thereafter solids were removed by centrifugation (10 min, 3000 g). 1 mL of the supernatants were added to 0.1 mL of the internal standard (2 g/L of 2-ethyl butyric acid), centrifuged at 10,000g and 4 °C for 15 min and 1 µL of this solution was injected into a Varian 3400 gas chromatograph (Varian Inc., Walnut Creek, CA) equipped with an injector at 170 °C, a flame-ionization detector at 175 °C, and a packed column (2 m × 2 mm i. d. glass containing 1–1965 10% SP-1200/1% H<sub>3</sub>PO<sub>4</sub> on 80/100 Chromosorb W). The temperature of the gas chromatograph oven was maintained at 140 °C. Gas flow rates were nitrogen, 40 mL/min and compressed air, 300 mL/min (Mirzaei-Alamouti et al., 2016).

### 2.4. Statistical analysis

Data analysis was performed using a completely randomized design. The GLM procedure of SAS (2003) was used for this purpose. Data of total VFAs and data of the reduction in the dry mass of potato wastes were subjected to statistical analysis as 2 × 2 factorial arrangements with the mass of the potato waste before fermentation and fermentation time as main factors by Eq. (3):

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk} \quad [3]$$

$Y_{ijk}$  represents the observation,  $\mu$  the overall mean,  $A_i$  the effect of the mass of potato waste before fermentation (100, 200, 300 or 400 gr per 200 mL of rumen fluid),  $B_j$  the effect of fermentation time (12 or 24 h),  $AB_{ij}$  the interaction and  $e_{ijk}$  the experimental error.

Data of nutrients composition and data of VFAs profile were analyzed as a completely randomized design by Eq. (4):

$$Y_{ijk} = \mu + A_i + e_{ijk} \quad [4]$$

$Y_{ijk}$  represents the observation,  $\mu$  the overall mean,  $A_i$  the effect of the mass of potato waste before fermentation (100, 200, 300 or 400 g per 200 mL of rumen fluid), and  $e_{ijk}$  the experimental error.

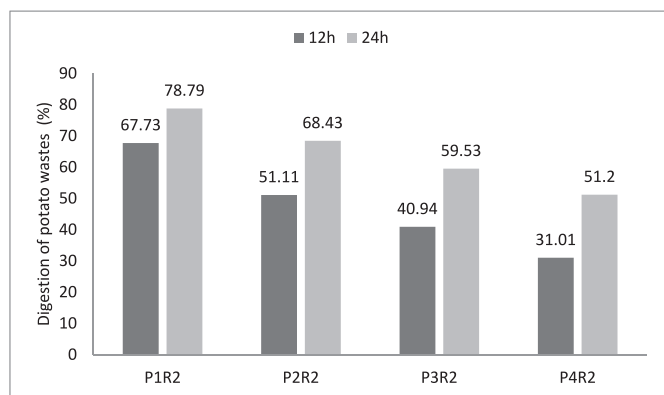
Duncan test was applied for the comparison between experimental groups. Significant differences were defined at  $P < 0.05$ .

## 3. Results

### 3.1. Digestion of potato waste

The reduction in the dry mass of the potato waste (solid phase of the fermentation mixture) by fermentation was considered as digested and expressed as the percentage of the dry mass of the potato waste before fermentation (Fig. 1). Fermentation time was shown to have a significant effect on the digestion extent of the potato waste. A fermentation time of 24 h resulted in a higher potato waste digestion compared to a 12 h fermentation irrespective the ratio of mass of potato waste to the volume of rumen fluid used ( $P < 0.05$ ) (Fig. 1).

The digestion of potato waste by rumen microorganisms present in the rumen fluid during fermentation was also significantly



**Fig. 1. Potato waste digestibility during fermentation (12 h, 24 h) at 39 °C in the presence of rumen fluid**

P1R2: ratio 1:2 of potato waste to rumen fluid, P2R2: ratio 2:2 of potato waste to rumen fluid, P3R2: ratio 3:2 of potato waste to rumen fluid, P4R2: ratio 4:2 of potato waste to rumen fluid. Digestion was considered as the reduction in the dry mass of the potato waste (solid phase of the fermentation) expressed as the percentage of the dry mass of the potato waste before fermentation.

affected by the ratio of mass of potato waste to the volume of rumen fluid. The highest digestion was observed at a ratio of 1:2 of potato mass to rumen fluid volume (P1R2) (Fig. 1).

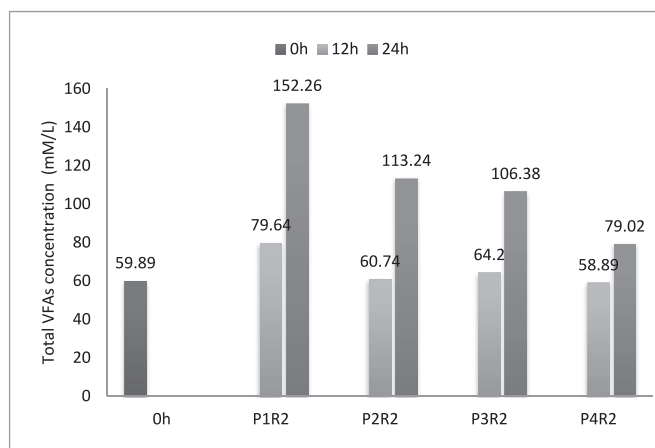
### 3.2. Total VFAs production

Fig. 2 shows the contents of total VFAs of the rumen fluid (the liquid phase of the fermentation mixture) after 12 and 24 h fermentation of the potato waste. Total VFAs content (including acetic, propionic, butyric, iso-valeric and valeric acid) increased by fermentation and longer fermentation time resulted in statistically significant higher total VFAs production ( $P < 0.001$ ). The content of VFAs was shown to decrease with an increase in the amount of potato waste used for fermentation for both fermentation times applied. The highest total VFAs content was obtained with ratio of P1R2 and a fermentation time of 24 h.

Since the 24 h fermentation time resulted in a higher digestion of the potato waste and in higher VFAs values compared to the 12 h fermentation time, only the 24 h fermented samples were used for further analysis.

### 3.3. VFAs composition of the liquid phase

Analysis of VFA composition of the liquid phase of the fermentation mixture revealed that its composition was affected by the fermentation process (Table 1). During fermentation, an increase in production of all VFAs quantified (acetic acid, propionic acid, butyric acid, iso-valeric acid and valeric acid) was observed. Acetic acid represented the volatile fatty acid with the highest concentration in the different fermentation mixtures as well as in the rumen fluid before fermentation. However, its relative proportion in the VFAs fractions decreased in favor of propionic acid by fermentation. As expected, the pH value of the liquid phase of the fermentation mixture dropped significantly during fermentation from almost neutral to about 4.0 ( $P < 0.001$ ) (Table 1) due to the production of the organic acids; the higher concentrations of the VFAs in the liquid fermentation phase the lower pH values observed.



**Fig. 2. Total content of VFAs (mM/L) of the rumen fluid (liquid phase of the fermentation mixture) after fermentation of potato waste in the presence of rumen fluid at 39 °C for 12 and 24 h.**

Control (total content of VFAs in the rumen fluid before fermentation): 0 h, P1R2: ratio 1:2 of potato waste to rumen fluid, P2R2: ratio 2:2 of potato waste to rumen fluid, P3R2: ratio 3:2 of potato waste to rumen fluid, P4R2: ratio 4:2 of potato waste to rumen fluid.

**Table 1**  
Content of the individual VFAs of the rumen fluid (liquid phase of the fermentation mixture) after fermentation of potato waste in the presence of rumen fluid at 39 °C for 24 h.

parameters	Control	P1R2	P2R2	P3R2	P4R2	SEM	P-value		
							M	L	Q
<b>VFAs (concentration, mM/L)</b>									
Acetic acid	41.43 <sup>e</sup>	96.81 <sup>a</sup>	74.79 <sup>b</sup>	70.62 <sup>b</sup>	52.45 <sup>d</sup>	1.36	<0.001	<0.001	0.15
Propionic acid	8.49 <sup>e</sup>	32.45 <sup>a</sup>	25.27 <sup>b</sup>	22.49 <sup>c</sup>	16.68 <sup>d</sup>	0.53	<0.001	<0.001	0.03
Butyric acid	5.68 <sup>e</sup>	15.14 <sup>a</sup>	9.67 <sup>b</sup>	8.72 <sup>c</sup>	6.54 <sup>d</sup>	0.13	<0.001	<0.001	<0.001
iso-Valeric acid	1.26 <sup>b</sup>	3.59 <sup>a</sup>	1.55 <sup>c</sup>	1.40 <sup>c</sup>	1.33 <sup>c</sup>	0.12	<0.001	<0.001	<0.001
Valeric acid	1.48 <sup>c</sup>	4.10 <sup>a</sup>	2.13 <sup>b</sup>	2.12 <sup>b</sup>	1.61 <sup>c</sup>	0.09	<0.001	<0.001	<0.001
Total VFAs	56.89 <sup>e</sup>	152.26 <sup>a</sup>	113.24 <sup>b</sup>	106.38 <sup>c</sup>	79.02 <sup>d</sup>	1.26	<0.001	<0.001	0.02
<b>VFAs (Fractional ratio, %)</b>									
Acetic acid	72.74 <sup>a</sup>	63.59 <sup>a</sup>	66.37 <sup>a</sup>	66.36 <sup>a</sup>	66.05 <sup>a</sup>	1.17	0.003	0.005	0.12
Propionic acid	14.92 <sup>b</sup>	21.30	22.32	21.16	21.11	0.43	<0.001	<0.001	<0.001
Butyric acid	9.98 <sup>a</sup>	9.94 <sup>b</sup>	8.54 <sup>c</sup>	8.29 <sup>c</sup>	8.19 <sup>c</sup>	0.13	<0.001	0.71	<0.001
iso-Valeric acid	2.22 <sup>a</sup>	2.67 <sup>b</sup>	1.24 <sup>c</sup>	1.46 <sup>c</sup>	1.67 <sup>c</sup>	0.16	0.37	0.002	<0.001
Valeric acid	2.60 <sup>ab</sup>	3.10 <sup>a</sup>	1.88 <sup>b</sup>	2.00 <sup>ab</sup>	2.05 <sup>ab</sup>	0.23	0.02	0.29	0.003
pH	6.95 <sup>a</sup>	3.89 <sup>c</sup>	4.03 <sup>bc</sup>	4.04 <sup>bc</sup>	4.15 <sup>b</sup>	0.05	<0.001	<0.001	<0.001

Different amounts of cooked and ground potato waste (100 g (P1R2), 200 g (P2R2), 300 g (P3R2) and 400 g (P4R2)) were fermented in the presence of 200 mL rumen fluid in a Daisy<sup>II</sup> incubator. Control: rumen fluid before fermentation. SEM: standard error of the mean. Effect of the dry mass of potato waste before fermentation: M: main effect, L: linear effect, Q: quadratic effect.

a-e Values within a row with different superscripts differ significantly at  $P < 0.05$ .

### 3.4. Nutrient composition of the solid phase

Fermentation of potato waste in the presence of rumen fluid also affected the nutrient composition of the solid materials remaining after fermentation (the solid phase of the fermentation mixture) (Table 2). The changes in the composition correlated well with the extent of the digestion of the potato waste during fermentation, thus with fermentation efficacy. Increasing the amount of potato waste in the fermentation mixture at a constant volume of rumen fluid resulted in a linear decrease in crude protein and ether extract content of the potato residuals ( $P < 0.001$ ). The highest crude protein and ether extract values were obtained with ratio 1:2 of potato waste to rumen fluid (P1R2) (Table 2). Compared to the crude protein content of the potato waste before fermentation, a 70% increase was observed after a fermentation time of 24 h. Neutral detergent fiber content, an indicator of the amount of structural carbohydrates present, was not significantly affected by fermentation (Table 2). The content of non-fibrous carbohydrates however, decreased significantly by fermentation ( $P < 0.001$ ). Furthermore, digestible organic matter and metabolizable energy were lower in residues remaining after fermentation compared to the potato waste used for fermentation ( $P < 0.001$ ) and could be explained by utilization of the digestible nutrients, especially non-fibrous carbohydrates, by rumen microorganisms. While increasing

the amount of potato waste in the fermentation mixture at a constant volume of rumen fluid, a decrease in digestible organic matter and metabolizable energy in the residues remaining after fermentation were observed ( $P < 0.001$ ).

## 4. Discussion

Cracked potatoes, damaged tubers obtained from harvesting, deformed and glassy tuber, under-sized tubers, and tubers injured by insects and pests are collected in potato depots and potato sorting factories and represent waste products. Management strategies for potato waste include its application as a fertilizer for cropland or as a feed for ruminants (Nelson, 2010). Because of the high moisture and carbohydrates content and the high contamination with microorganisms, potato waste is sensitive to spoilage and has a low storability. Therefore, potato waste does not represent a preferred feed component nor is its use profitable. The aim of the present study was to valorize potato waste by fermentation using rumen fluid, a further waste product. Millions of ruminant animals, including cattle, sheep, goat, camel and buffalo, are slaughtered worldwide every day and their rumen fluid is released into the environment causing pollution (Rezai-Sarteshnizi et al., 2018). The enormous potential of the rumen microbiota as a source of enzymes was already recognized and rumen

**Table 2**  
Effect of a 24 h fermentation of potato waste in the presence of rumen fluid on the nutritional composition (% DM) of the solid residues of the fermentation mixture.

parameters	Control	P1R2	P2R2	P3R2	P4R2	SEM	P-value		
							M	L	Q
CP	10.11 <sup>d</sup>	17.95 <sup>a</sup>	14.45 <sup>b</sup>	13.52 <sup>bc</sup>	12.92 <sup>c</sup>	0.26	<0.001	<0.001	0.110
EE	3.67 <sup>b</sup>	5.67 <sup>a</sup>	4.33 <sup>ab</sup>	4.00 <sup>b</sup>	3.67 <sup>b</sup>	0.30	0.004	0.006	0.040
NDF	15.23	16.40	13.80	17.30	15.54	1.14	0.330	0.870	0.880
NFC	65.66 <sup>a</sup>	51.22 <sup>c</sup>	58.82 <sup>ab</sup>	57.60 <sup>bc</sup>	58.94 <sup>ab</sup>	1.57	0.001	0.002	0.900
Ash	5.33 <sup>c</sup>	8.76 <sup>a</sup>	8.59 <sup>a</sup>	7.58 <sup>b</sup>	8.94 <sup>a</sup>	0.21	<0.001	<0.001	0.002
DOM	59.65 <sup>a</sup>	40.63 <sup>d</sup>	44.98 <sup>c</sup>	45.40 <sup>c</sup>	48.89 <sup>b</sup>	0.88	<0.001	<0.001	<0.001
ME (MJ/Kg DM)	9.03 <sup>a</sup>	6.05 <sup>d</sup>	6.70 <sup>c</sup>	6.77 <sup>c</sup>	7.30 <sup>b</sup>	0.15	<0.001	<0.001	<0.001

Different amounts of cooked and ground potato waste (100 g (P1R2), 200 g (P2R2), 300 g (P3R2) and 400 g (P4R2)) were fermented in the presence of 200 mL rumen fluid in a Daisy<sup>II</sup> incubator. Control: potato waste before fermentation. SEM: standard error of the mean. Effect of the dry mass of potato waste before fermentation: M: main effect, L: linear effect, Q: quadratic effect. CP (crude protein), EE (ether extract), DM (dry matter), NDF (neutral detergent fiber), NFC (non-fibrous carbohydrates), DOM (digestible organic matter) and ME (metabolizable energy).

a-d Values within a row with different superscripts differ significantly at  $P < 0.05$ .

microorganisms have been shown to be able to digest even biomass containing lignin (Barnes and Keller, 2004). In this study, it was shown that the microorganisms present in the rumen fluid were able to digest amylosic potato waste. A reduction of up to 78.8% of the dry mass of the potato wasted was observed during fermentation (Fig. 1) after a fermentation time of 24 h. Volatile fatty acids (VFAs) were reported to be the main products of anaerobic digestion of agricultural waste (Wang et al., 2018; Zhang et al., 2016). The target products at present study was organic acids including acetic, propionic, butyric, iso-valeric and valeric acids, called volatile fatty acids. The study aimed to generate VFAs from potato waste as a substrate by microorganisms present in the rumen fluid. It was already reported that the rumen fluid contains acidogenic bacteria capable of producing VFAs by degrading or decomposing plant material (Yue et al., 2013). Besides fermentation time, the relative amount of potato waste used for fermentation was shown to affect VFAs production. Acidogenic bioconversion was shown to have a certain optimum in respect to substrate turnover. Below the optimum substrate concentration, the available substrate is the limiting factor and above the optimum substrate concentration substrate degradability was identified as the limiting factor (Sanchez et al., 2001). Increasing the concentration of the potato waste by a factor of 4 resulted in a decrease in total VFAs concentration from 152.26 mM/L to 79.02 mM/L (Fig. 2) applying a fermentation time of 24 h. Thus, the optimum in potato waste concentration in respect to digestibility seems to be below 100 g potato waste per 200 mL rumen fluid. High yields in VFAs have already been reported with agricultural residues as substrates for acidogenic microorganisms (Barnes and Keller, 2004; Parawira et al., 2005). Concentrations of total VFAs up to 320 mM/L were achieved after 10 days of incubation (Parawira et al., 2005). In present study, up to 152.26 mM/L were obtained within 24 h. As shown in Table 2, the taken rumen fluid from the slaughterhouses contains initially of 56.89 mM total VFAs. Yue et al. (2013) already proposed to use rumen fluid directly as a source of VFAs. For applications, they suggested an extraction or concentration process. Since the liquid phase obtainable after fermentation contains higher concentrations of VFAs, it represents even a more suitable source for extraction or concentration processes. VFAs such as acetic, propionic, butyric and lactic acids have been used extensively as dietary acidifiers in animal and poultry nutrition. Acetic and propionic acids represent commercial acidifiers due to their pH-lowering effects in the gastrointestinal tract, thereby suppressing the colonization of the gastrointestinal tract by pathogens (Khan and Iqbal, 2016). Butyric acid was shown to exhibit beneficial effects on rumen development when fed to neonatal suckling ruminants (Gorka et al., 2011). Supplementing feed with for example valeric or iso-valeric acid was reported to support the growth of cellulolytic bacteria in the rumen resulting in a better fiber digestion by the animals (Andries et al., 1987). However, also the VFA-enriched rumen fluid obtainable by fermentation might be applied in animal feeding. Besides VFAs, rumen fluid contains different other nutrients such as microbial proteins, amino acids, vitamins and minerals (Yue et al., 2013). It was already shown that fresh, autoclaved or centrifuged rumen fluid had a positive effects on weight gain and diarrhea incidence when used as a feed additive for suckling calves (Muscato et al., 2002). Furthermore, Rezai-Sarteshnizi et al. (2020) reported a positive effect on suckling calves' immune system when feeding spay-dried rumen fluid.

The relative concentration of the different volatile fatty acids within the VFAs fraction depends on the type of carbohydrate used for fermentation by rumen microorganisms. Acetic acid is in general the main volatile fatty acid produced; however, the relative concentration of propionic acid will increase when amylosic substrates are fermented (McDonald et al., 2011). The relative concentration of propionic acid increased at the expense of acetic acid

when amylosic potato waste was fermented by rumen microorganisms.

The fermentation process resulted in higher protein and fat contents as well as in lower metabolizable energy and digestible organic matter contents of the solid residues remaining after fermentation. These results are in good agreement with observations reported by Aziz and Mohsen (2002). They found an increase in protein content after fermentation of sweet potato residues using *Fusarium moniliforme* and *Saccharomyces cerevisiae*. In addition, significant increases in crude fat, crude protein and energy contents accompanied by reductions in the crude fiber contents were reported by Onyimba et al. (2014) when treating sweet potato leaves and sorghum grains with *Aspergillus niger*, *Chaetomium globosum* or *Saccharomyces cerevisiae*. The observed increase in the protein contents could be easily explained conversion of plant-derived carbohydrates to microbial proteins during fermentation. The lower digestible organic matter contents and the lower metabolizable energy of the solid residues remaining after fermentation are also in agreement with this explanation. Due to their high protein contents, the solid residues remaining after fermentation could be used as a protein source in the diet of farm animals.

While fermenting agricultural waste by rumen microorganisms in a bioreactor, controlling the pH value of the reactor content is in general of utmost importance. Due to the production of organic acids, the pH of the reactor content drops significantly and at pH values below 6.0 cellulolytic bacteria are not capable of digesting cellulose anymore (Russell and Wilson, 1996). In present study, amylosic waste was used for fermentation and digestion rates up to 78.8% were achieved without any pH control. Due to the production of organic acids during fermentation the pH of the bioreactor content dropped from pH 6.95 to pH values around 4.0. Therefore, the amylolytic bacteria present in the rumen fluid are not sensitive to low pH values. Thus, the developed approach to valorize both potato waste and rumen fluid is simple, only temperature needs to be controlled and a proper level of potato waste chosen. Thus, the process has the potential for be scaled-up.

## 5. Conclusion

A simple fermentation process to valorize the two waste products, potato waste and rumen fluid, was developed. Optimal conditions in respect to the production of volatile fatty acids in the fermentation process and the content of crude protein in the solid phase remaining after fermentation have been found using 100 g of potato waste per 200 mL of rumen fluid (ratio 1 to 2 of potato waste mass to rumen fluid volume) and a fermentation time of 24 h. A crude protein content of 17.95% in the solid phase remaining after fermentation and a production of 92 mM/L of total VFAs resulting in a VFAs concentration of 152 mM/L in the liquid phase remaining after fermentation was achieved. Both products, the protein-enriched solid phase and the VFA-enriched liquid phase, might be suitable animal feed additives. The develop approach contributes therefore in the reduction of agricultural wastes and in a reduction in the environmental pollution caused by the two waste products. The process only needs temperature control and a proper level of potato waste. Therefore, the process has the potential to be scaled-up.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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