Screening of starter cultures for oat-based non-dairy yoghurt

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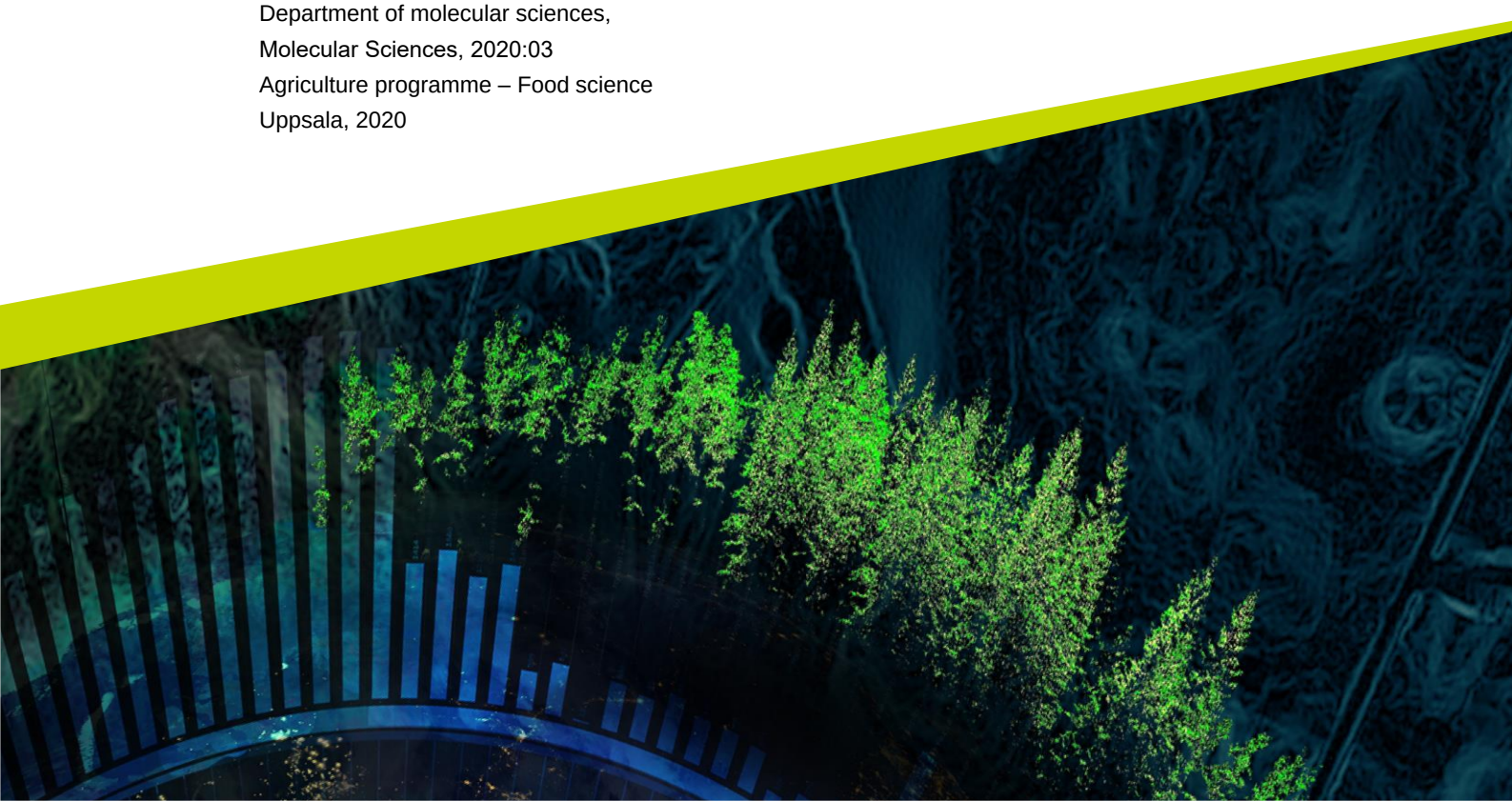
Master thesis • 30 hp
Swedish University of Agricultural Sciences, SLU
Department of molecular sciences,
Molecular Sciences, 2020:03
Agriculture programme – Food science
Uppsala, 2020



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Credits:	30 hp
Level:	A2E
Course title:	Master thesis in food science
Course code:	EX0877
Programme/education:	Agriculture programme - Food Science
Course coordinating dept:	Department of molecular sciences
Place of publication: Year of publication:	Uppsala 2020
Title of series:	Molecular Sciences
Part number:	2020:03

Keywords:	starter cultures, plant-based yoghurt, oats, LAB, fermentation, non-dairy, plant protein

Swedish University of Agricultural Sciences
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Abstract

In this project a selection of different starter cultures was used to ferment oat-based yoghurt made with different plant proteins. The fermentation capacity of the starter cultures was investigated by continuous monitoring of the pH during the fermentation process of the yoghurts. Furthermore, the ability of each starter culture to use different energy sources was investigated as well as their ability to produce lactic acid in combination with each protein. A sensory profile test was carried out to determine some chosen sensory attributes of the yoghurts as well as to categorize the starter cultures.

The results from this screening shows that both different starter cultures and a specific starter culture in combination with different proteins yields different end products. The starter culture used in plant-based fermentation should thus be selected with the food matrix in mind. The traditional yoghurt starter cultures used in this screening gave rise to yoghurts with general higher pH and lower amounts of lactic acid. They were also sensory described as sweet and lacking in acidity.

Starter culture G in combination with protein 17, 10, 11 and 3 gave rise to the most acidic yoghurts, while starter culture H in combination with protein 17, 16, 10 and 3 gave rise to the yoghurts which were perceived as most sweet.

The results from this screening may be seen as a database of different starter cultures in combination with different plant proteins. It could be further extended to include other starter cultures as well as other proteins. The results can be used as a starting point in product development of new plant-based fermented products.

Keywords: starter cultures, plant-based yoghurt, oats, LAB, fermentation, non-dairy, plant protein

Sammanfattning

I detta projekt har ett antal olika starterkulturer använts för att fermentera havre-baserad yoghurt med tillsats av olika växtprotein. De olika starterkulturernas benägenhet till att fermentera yoghurten undersöktes genom att kontinuerligt mäta pH-värdet under fermenteringsprocessen. Vidare undersöktes även starterkulturernas benägenhet till att använda olika energikällor och mängden mjölksyra som bildades under fermenteringen med de olika växtproteinerna. Ett sensorisk profiltest utfördes för att bedöma några utvalda attribut i yoghurten för att kunna kategorisera starterkulturerna utefter dessa.

Resultatet från denna screening visar att både olika starterkulturer och varje enskild starterkultur i kombination med olika växtprotein ger upphov till olika slutprodukter. Starterkulturer till fermentering av växtbaserade produkter skall därför väljas med omsorg för produkten de skall fermentera. De traditionella yoghurt starterkulturerna som användes i denna screening gav generellt sett yoghurt med högre pH-värde och lägre mängd mjölksyra. De beskrevs även sensoriskt som söta och saknade syra.

Starter kultur G i kombination med protein 17, 10, 11 och 3 gav upphov till de yoghurts som beskrevs som syrligast. Starter kultur H i kombination med protein 17, 16, 10 och 3 gav upphov till de yoghurts som beskrevs som sötast.

Resultatet från denna screening kan användas som en databas över olika kombinationer av starter kulturer och växtproteiner och kan vidareutvecklas till att inbegripa fler kombinationer. Resultatet kan användas som utgångspunkt i produktutveckling av nya växtbaserade fermenterade produkter.

Nyckelord: starterkulturer, växtbaserad yoghurt, havre, LAB, fermentering, mejerialternativ, växtprotein

Table of contents

List of tables

List of figures

Abbreviations

LAB	Lactic acid bacteria
TTA	Total titratable acids

1. Introduction

The continuous global production increase of plant-based non-dairy products reflects the increasing consumer demand for plant-based dairy alternatives (Sumesh & Roshan, 2019). The demand was initially driven by people with milk intolerance but plant based milks are increasingly being consumed as healthy alternatives to milk products (Intelligens, 2006). Soy milk is the most commonly used plant based milk alternative, but have been declining on the market for several reasons, one being the competition from other plant milks, such as oats, almonds and coconut (Mäkinen *et al.*, 2015; Mintel, 2011).

The shelf life of plant-based milks may be extended by fermentation with lactic acid bacteria (LAB). The LAB may also improve texture, nutritional value and sensory attributes of the plant milk (Leroy & De Vuyst, 2004). Several previous studies have concluded that oats, including oat milk, are good growth substrates for LAB. The LAB used for oat fermentation in previous studies are mainly the traditional yoghurt producing species; *Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptoococcus salivarius* subsp *thermophilus* as well as *Lactobacillus acidohpilus* and *Bifidobacterium*, both as single and mixed cultures (Brückner-Gühmann *et al.*, 2019; Mårtensson *et al.*, 2002; Mårtensson *et al.*, 2001).

Fermentation of oats seem to have a great impact on the formation of flavour active volatiles (Salmeron et al., 2009). The development of different flavours depends on the starter culture used and what substrate its grown in (Salmerón, 2014). *Lactobacillus brevis, Lactobacillus kefiri, Pediococcus damnosus, Propionibacterium propioniacidici, Leuconostoc mesenteroides and Leuconostoc dextranicum* have also exhibited good fermentation capacity in oat milk but with varying sensory acceptance (Mårtensson *et al.*, 2000). Consumer acceptance is dependent on the sensory quality of a product (Yuceer & Drake, 2007). Choosing the right combination of starter culture and substrate would therefore seem important when developing new plant-based fermented products with sensory acceptance. The need for further and deeper investigations of LAB suited for fermented plant products, both regarding technological and sensorial aspects seem evident.

In contrast to traditional yoghurt and starter cultures for dairy products, there is limited research about starter cultures suitable for plant-based products (Gu *et al.*, 2020; Tian *et al.*, 2019; Baran *et al.*, 2012; Soomro & Masud, 2008). The traditional

starter cultures used for dairy products may not be the most optimal for plant-based products. Thus, this project aimed to screen for starter cultures suited for plant-based fermentation. This was investigated by fermenting non-dairy oat-based yoghurts with different starter cultures. The influence of the starter cultures in combination with different added plant proteins in the yoghurts was studied. This work was carried out for Oatly AB (Landskrona, Sweden) using oat base obtained from the production as the main ingredient of the yoghurts. The fermentation capacity of the starter cultures was studied by measuring pH in-line and the amount of titratable acids forming during the fermentation process. The live counts of the starter cultures before and after fermentation was also determined. Finally the suitability of the starter culture for oat fermented products was sensory evaluated by conducting a sensory profile test of the most promising starter cultures.

1.1. Yoghurt

Streptococcus thermophilus and *Lactobacillus delbrueckii* sups. *bulgaricus* are the two traditionally LAB used for dairy yoghurt production. The milk is usually supplemented with solids up to between 11-15 % prior to fermentation. The fermentation is carried out at 42° C (the optimal temperature between the two species) during 4 hours a pH between 3,8-4,2 is reached as a results from the LAB's production of lactic acid from lactose. Acetaldehyde is the most important volatile compound produced by the LAB and should be present at 23-41 mg/kg $^{-1}$ for producing the typical yoghurt flavour. Diacetyl is another flavour volatile which also contributes to the flavour (Adams & Moss, 2008).

1.1.1. Gel structure

In dairy yoghurt, the casein micelles in the milk aggregates to form a gel as a result of lowered pH due to LAB's production of lactic acid during the fermentation (Adams & Moss, 2008). The gelling properties for plant-based proteins works different compared to the casein in milk. A previous study found pea protein to be weaker compared to soy proteins and the gelling is dependent on sufficient heating (Tulbek *et al.*, 2016). Another study found an optimal gelling condition with pea protein using a protein content as high as 19,6 % (Shand *et al.*, 2007). A previous study reported low or no protein content in 17 different commercial plant milks (Jeske *et al.*, 2017). The low gelling properties as well as the low protein content in plant-based milks poses a problem if the plant milk is to be used to make a plant-based yoghurt.

In fermented yoghurts based on oat fraction and oat concentrate it has been reported that starch rather than protein seem to make up the gel structure (Bruckner-Guhmann *et al.*, 2019; Loponen *et al.*, 2007). This is probably due to the low

solubility in oat protein compared to other plant proteins, especially between pH 4-7 which is the typical range for foods (Mäkinen *et al.*, 2017). Plant-based non-dairy yoghurt alternatives seem therefore to be dependent on starches building up a gel structure compared to traditional yoghurt where proteins together with bacteria are the main influences of the texture. Some LAB have the ability to produce glycoprotein slime or exopolysaccharides (EPS), which may provide a ropy texture and enhance the viscosity of the yoghurt. They may be of importance when it comes to enhance the viscosity of a plant-based yoghurt (Adams & Moss, 2008).

1.2. Lactic acid bacteria

The group Lactic acid bacteria (LAB) includes a number of bacteria such as *Lactobacillus*, *Streptococcus*, *Lactococcus*, *Pediococcus*, *Leuconostoc* and *Bifidobacterium*. LAB are gram positive, non-spore forming rods or cocci-shaped bacteria. They have a long history of being used in food applications in the production of cheese, yoghurts, fermented vegetables, fish or meat and in bread making (Bintsis, 2018). They are naturally occurring in a variety of habitats such as on plants and plant material, mucosal membranes of humans and animals, manure, sewage systems and in fermenting and spoiling foods (Hammes & Vogel, 1995).

In foods, LAB are often inhibitory to other microbes mainly due to their ability to produce lactic acid, which lowers the pH of the product, and antimicrobial components (Adams & Moss, 2008). Additionally, LAB contributes to flavour, texture and nutritional value by degradation of sugars, lipids and proteins. Sugar is needed as an energy source in order for LAB to grow. Homo-fermentative LAB produces lactic acid as the main end-product of metabolism while hetero-fermentative LAB produces other end products such as CO_2, ethanol and acetic acid in addition to the lactic acid. Some LAB have the ability to use both metabolic pathways.

Commercial starter cultures are used extensively within the food industry in contrast to the traditional method of back-slopping, where a new product was inoculated with the product from previous day. The use of starter cultures assures an even product quality in a more automated food process, where larger quantities with total control over the process is demanded (Bintsis, 2018).

When inoculating a medium with a starter culture the bacteria encounter an environmental chock. During this period, called lag-phase, there is no bacterial growth as the bacteria is adjusting to the new environment, synthesizes enzymes required to grow and repairs any injuries from previous handling. The length of the lag phase depends on the type of bacteria, the age and size of the inoculum and changes of the nutritional composition, pH and temperature (Bintsis, 2018;

Kampen, 2014). The exponential phase, in which the bacteria grows and increase in cell numbers, follows the lag phase. The stationary phase is initiated when the growth naturally stabilizes due to changes in the media, such as low pH, nutrition depletion and the accumulation of inhibitory metabolites. (Adams & Moss, 2008).

Commercially available freeze-dried mixed starter cultures have been used in this project. The starter cultures contain between 2-5 different species and have been developed by the starter culture industry for fermentation of plant-based products. An overview of the general ability to ferment glucose, sucrose and maltose for the species included in the starter cultures may be seen in Table 1. The ability of the bacteria to ferment the different sugars may differ between bacterial strains (Hedberg *et al.*, 2008).

Table 1. A summary of LAB used in this project and their ability to ferment sucrose, glucose and maltose

Bacteria	Glucose	Sucrose	Maltose	Reference
Lactobacillus delbrueckii subsp. *bulgaricus (L.del.bulgaricus)*	+	-	-	(Ceapa *et al.*, 2015; Farnworth, 2008; Hedberg *et al.*, 2008; Hammes & Vogel, 1995)
Lactobacillus delbrueckii subsp. *lactis (L.del.lactis)*	+	+	+	
Lactobacillus acidophillus (L. acidophillus)	+	+	+	
Lactobacillus paracasei (L. paracasei)	+	+	-	
Lactobacillus plantarum (L. plantarum)	+	+	+	
Lactobacillus rhamnosus (L.rhamnosus)	+	+	-	
Bifidobacterium animalis subsp. *lactis (B.ani. lactis)*	+	+	+	(Pokusaeva *et al.*, 2011; Ruas-Madiedo *et al.*, 2005)
Bifidobacterium animalis (B.animalis)	+	+	+	(Fritsch *et al.*, 2015)
Pediococcus pentosaceus (P. pentosaceus)	+	-	+	(Simpson & Taguchi, 1995)
Streptococcus thermophilus (St. thermophilus)	+	+	-	(van den Bogaard *et al.*, 2004; Hardie & Whiley, 1995)

2. Materials and methods

The following laboratory work was carried out at Oatly AB in Landskrona, Sweden. The oat base used in the production of the yoghurts in this project was obtained directly from the production. The plant proteins and starter cultures used were from different sources and brands and have been coded based on the confidential nature of this study. The recipe used for the yoghurts have been generalized based on its confidential nature.

2.1. Oatbase

The oat base is made by wet milling oats with hot water. An enzymatic reaction involving β-amylase yields maltose and β-limit dextrins as primary carbohydrates derived from the starch. After the enzymatic hydrolysis, insoluble fibers are separated and the oat base is heat treated. The oat base used in this project had a final dry matter content of 12,73-14,14 %.

2.1.1. HPLC analysis

The sugars in the oat base were determined by HPLC. 3 ml oat base from each batch used for the yoghurts were centrifuged at 13,4 *1000 rpm for 10 min. 1 ml of the supernatant was diluted with 5 ml distilled water. The solution was filtered through a 0,2 µm filter into HPLC vials. The HPCL was run with pre colon Zorbax Analytical Guard column, 4,6 *12,5 mm, 5 µm particles (Agilent technologies) prior to Zorbax Carbohydrate Analysis Column, 4,6 *150 mm, 5 µm particles (Agilent Technologies). Sucrose, glucose, maltose and maltotriose were used as standards.

2.2. Starter cultures

Seventeen different starter cultures (A-Q) were evaluated for this project. The selection included thirteen starter cultures developed by the starter culture industry for plant-based fermentations and four traditional starter cultures (coded H, M N, O) used for yoghurt production (*L. bulgaricus* and *S. thermophilus*).

2.3. Initial screening of 15 different starter cultures and seven plant proteins

2.3.1. Plant proteins

A selection of different plant proteins (see Table 3, section 3.2.2) was diluted in oat base to gain a 4 % protein content. These were sensory evaluated by four individuals with good product knowledge at Oatly. The samples were described and scored 1-3. Score 1 was used for samples not suitable for yoghurt application, 2 was used for samples with some potential and 3 was used for samples with very good potential for yoghurt application. Seven proteins were selected for the yoghurt production based on their sensory attributes. Important attributes of the selection were overall taste, texture and colour as well as protein content and solubility. The grainy texture of some of the proteins was expected to disappear after homogenization and thus not considered during the selection.

2.3.2. Production of oat yogurt

All ingredients were weighed in according to the generalized recipe in Table 2.

Table 2. General recipe of the yoghurt

INGREDIENTS	PERCENTAGES
Oil	3 %
Sugars	1 %
Protein	3 %
Starch	3 %
Oat base	90 %

Oatbase was heated to 60 °C in a Thermomixer (Thermomix TM6, Vorwerk, Germany). Sugars (sucrose was used for all yoghurts) and starch were added followed by the protein. After 5 minutes of continuous mixing, the oil was slowly added. The solution was sifted (500 µm) prior to heating it up to 70°C. The homogenizer (Lab homogenizer Twinpanda 600, GEA mechanical equipment IT S.p.A, Parma, Italy) was heated to 70 ° by slowly increase the temperature of the water passing through. To increase the stability and viscosity as well as to make the yoghurts appear whiter, the yoghurt mixture was homogenized at 250/50 bar. After pasteurization (95 °C, 2 minutes) in the Thermomixer, the solution was cooled

down in room temperature for 30 minutes and then transferred to sterile 1000 ml beakers under sterile conditions. The solution was cooled to 38 ° before 100 ml was distributed in 15 sterile beakers.

Freeze-dried starter cultures (A-O) were equilibrated in room temperature for 15 minutes prior to solving 0,5 g culture in 50 ml autoclaved 0,9 % NaCl under sterile conditions. Each 100 ml yoghurt was inoculated with 1 ml culture and incubated overnight (16-18 h) at 38 °C and then stored in 6° C until further analysis.

2.3.3. Measuring pH and Total Titratable Acids

The pH of the yogurts was measured (Sartorius Basic meter PB-11, Sartorius mechatronics, Germany) before and after fermentation to ensure a proper fermentation and thus a microbial safe product.

Total titratable acids (TTA) were determined by titrating 1 M NaOH into 20 ml yogurt mixed with 3 drops of o-cresolphthalein 20 g/l in ethanol 70%. When needed, distilled water was used to dilute the sample. The amount NaOH needed for a visible colour change was measured as the difference in weight (g) of added NaOH before and after titration. Six commercially bought plant-based non-dairy yoghurts as well as one traditional yoghurt was also measured.

2.3.4. Sensory characterisation of yoghurts

Five females (ages 27-60) evaluated the yoghurts during the initial screening by tasting them and describe each of them with descriptive words. The assessors were chosen on the basis of their good product knowledge for oat products. The tasting was carried out during several sessions, tasting between 8-16 yoghurts/session. The assessors were asked to consume sparkling water and crackers between each sample. Each yoghurt was also rated in the same way as done with the proteins.

2.4. In-line pH measurements

The fermentation process (pH values and temperature) was monitored in-line every four minutes using iCinac (AMS alliance, Rome, Italy) during 16 hours in a 38° C shaking water bath. The fermentation process is presented in a diagram in which the pH decrease is plotted against the time. The iCinac allows for different features to be applied to the data analysis. The Log phase (measured as time until ΔpH = 0,08 which is the standard lag phase used within the dairy industry), Time to reach pH 4,3, End pH after 16 hours, Mean temperature, Max acidification rate and Time at max acidification rate was measured during the fermentation process. The lag phase, Max acidification rate and Time at max acidification rate gives information about how fast the fermentation will proceed and how well the starter culture does in the food matrix. Time to reach pH 4,3 and end pH after 16 h gives similar

information but on a more general level. Mean temperature was logged to ensure an even and correct temperature during the whole fermentation. Other features are possible to apply in the iCinac program.

2.4.1. Fermentation with different starter cultures and proteins

Pasteurized, homogenized oat yoghurt mixture with protein 3, 10, 11, 14, 16 and 17 was inoculated with starter cultures A, B, C, D, E, F, G, H, I, P and Q. The fermentation process was monitored in-line with iCinac during 16 h.

2.4.2. Fermentation with different energy sources

Pasteurized, non-homogenized oat base was inoculated with starter cultures (A-Q, except K which was out of stock). One batch was made with only sucrose, one with only glucose and one with only maltose as the added energy source. The fermentation process was monitored in-line with iCinac during 16 h.

2.5. Monitoring of live bacteria

M17 (selective for Streptococci) and MRS (selective for Lactobacillus) agar plates were made accordingly to the instructions on the package. 1 ml of diluted starter culture (0,5 g in 50 ml 0,9 % NaCl) used for inoculating the yoghurt mixtures was diluted to -7 using dilucups (Dilucup Elegance BPW) and Dilushaker (Labrobot, Stenugnsund, Sweden). 1 µl of dilutions -5, -6 and -7 was spread onto MRS agar and M17 (only starter cultures A, B, H and I).

After fermentation of the yoghurts, 1 ml of each yoghurt was diluted and spread onto agar plates with the same procedure as for the starter cultures. All plates were incubated anaerobically at 36 °C during 2 days before the colonies were counted.

2.6. Sensory descriptive test

Five to six assessors (due to COVID-19 these were not the same as in previous sensory characterization nor the same between each session) were engaged in descriptive tests of yoghurts made with the six different proteins (3, 10, 11, 14, 16, 17) in combination with starter cultures A, B, C, D, E, G, H, I. The intensity of six attributes chosen from the initial screening of yogurts was individually evaluated on a 10 cm scale. The attributes were chosen on the basis of being discriminative between the samples and being used in the initial sensory characterization, since the time to train the assessors was limited. The attributes were; Acidity, Sweetness, Creaminess (including words used in the initial sensory screening such as creamy and full body), Fruitiness (including flavor of fruits and berries), Taste of protein

(including the taste of different pulses, grains and tubers) and Off-flavour (including words used by the previous panel such as yeast, stale and carton).

The samples were presented blind, simultaneously and randomly coded with three-digit numbers. To minimize physiological and psychological errors as described by Meilgaard & Carr the serving order for each assessor was also randomized (Meilgaard & Carr, 2006). The assessors were asked to consume sparkling water and crackers in between each sample. After the individual evaluation, a discussion which aimed to reach consensus between the assessors was carried out. The consensus discussion about each sample and its intensity yielded one common rating per attribute and sample. The descriptive tests were carried out during six sessions, one protein per session.

3. Results

3.1. Sugar composition of the oat base

The HPLC-analysis confirmed maltose to be the main sugar in the oat base together with very low concentrations of sucrose and glucose. The result showed an average concentration of 0,40 g/l glucose, 1,62 g/l sucrose, 44,84 g/l maltose and 13,62 g/l maltotriose.

3.2. Initial screening of 15 different starter cultures and seven plant proteins

3.2.1. Screening of plant proteins

Nineteen different commercially available plant proteins were evaluated during the initial screening. Soy and nut proteins were not included. The plant proteins included were isolates and derive from pulses, tubers and grains. The protein was added to the yoghurts with the aim to increase the protein content to equivalent levels as for a dairy yoghurt. Isolates were chosen over concentrates since isolates contains a higher protein content (González-Pérez & Arellano, 2009).

Seven plant proteins were selected as best suited for yoghurt application based on the sensory perception during the initial screening. Protein 17, 10, 16, 12, 3, 14 and 11 (marked in bold in Table 3) were all highest rated and described as the mildest versions within their respective plant protein source. It was also noted that protein isolates derived from the same plant source but from different brands had different taste and appearance.

Table 3. Summary of the proteins used and their protein content. The descriptive words and their rating generated from the tasting session are shown. The proteins market in bold are the ones used for the yoghurt production.

PLANT PROTEIN SOURCE	PROTEIN CONTENT %	DESCRIPTIVE WORDS	RATING
13	45	Mild, butterscoth, burnt flavor. Not suitable for yoghurt	1
17	**90**	**Mild, sweet, beany flavor, mild acidity, neutral**	**3**
10	90	Very Mild, mild beany flavour, neutral, creamy	3
10	**90**	**Mild, mild beany flavor, neutral**	**3**
10	80	Bitter, sour, off-flavour, tastes of pulses	2
16	60	Mild, tasteless, bitter	1,5
16	**88**	**Mild, tasteless, a little bitter, beany flavour, off-flavour, neutral**	**2**
3	80	Mild, sweet, full-bodied, neutral, taste of pulses, fresh	2,5
3	85	Bitter, sweet, not fresh, grassy flavor, bitter	1
3	84	Grassy flavor, taste of pulses, earthy, vegetative, bitter, not fresh, rancid	1
12	**50**	**Mild, bitter, neutral, tastes rancid, taste of carton and paper, salty**	**1,5**
3	90	Bitter, salty, metallic taste	1
3	85	Mild, sweet, suitable for yoghurt, tastes pulses, bitter, neutral, taste of flour	2
3	**85**	**Mild, sweet, full-bodied, creamy, neutral, yeast flavour, fruity, suitable for yoghurt**	**2,75**
3	85	Mild, sweet, chalky, off flavor, neutral	2,5
3	86	Bitter, smoky, sweet, mild, not fresh	1
14	**90**	**Very acidic, off flavours**	**1,5**
11	**80**	**Taste of pulse, spicy, taste of paper, off-flavour, flavour stays for a long time**	**1,5**
11	80	Strong flavour, taste of pulse, spicy	1

3.2.2. pH and Total Titratable Acids measurements

The pH and TTA were measured before and after 16 hours fermentation in 38°C. The pH value varied between 3.66 – 5.50 among the yoghurts as seen in Table 4. The yogurts containing protein 12 had a generally higher pH compared to the other proteins.

Starter cultures H and K-O measured a generally higher pH compared to the other starter cultures.

Table 4. pH values for each yoghurt before and after 16 h of fermentation at 38° C. pH-values below 4,3 are marked red.

	17	10	3	14	12	16	11
INITIAL PH BEFORE FERMENTATION	7,34	7,34	7,16	6,15	6,45	7,33	7,29
STARTER CULTURE							
A	3,95	4,28	4,02	3,68	4,32	4,04	3,99
B	3,82	4,08	3,88	3,66	4,19	3,86	3,86
C	3,70	3,91	3,89	3,96	4,09	3,74	3,74
D	4,19	3,86	3,89	3,89	4,14	3,84	3,87
E	3,89	4,17	4,00	3,90	4,59	3,96	4,03
F	4,51	3,80	3,90	3,71	4,46	3,89	3,85
G	3,71	4,58	3,83	3,73	4,19	4,40	3,93
H	4,13	4,42	4,40	4,47	5,19	4,27	4,30
I	3,86	3,95	3,97	4,20	4,83	3,97	3,99
J	3,83	3,90	3,83	4,36	3,90	3,87	3,91
K	4,26	4,16	4,21	5,50	4,38	4,02	4,14
L	3,99	4,18	4,55	4,59	4,16	4,02	4,06
M	4,13	4,60	4,07	4,65	4,42	4,27	4,30
N	4,35	4,98	4,47	4,65	4,93	4,40	4,39
O	4,24	4,72	4,47	4,25	4,50	4,39	4,44

Compared to pH, TTA is a better indicator of acids impact on flavor in foods. TTA includes all acids derived from Krebs cycle and their derivatives as well as fatty acids and amino acids but since titration cannot distinguish between these, TTA is usually used interchangeably as the predominant acid (Sadler & Murphy, 2010). On this basis TTA measurement was carried out as an indicator of the presence of lactic acid.

Protein 3 and 11 generally contained a higher amount of TTA compared to the other proteins while proteins 17 and 14 exhibited lower amounts of TTA than the rest (see Table 5). Starter cultures H and K-O measured the lowest amount of TTA, which correlates with the higher pH of these starter cultures.

Six non-dairy plant-based yoghurts found at the market were used as references. The amount of NaOH used for these were found to be between 1,11-2,1. A dairy yoghurt was also tested as a reference and needed 2,51 ml NaOH for a visible color change. Only one yoghurt with protein 16 fermented with starter culture C reached a value higher than 2,00 ml NaOH in this screening.

Table 5. Total titratable acids (TTA) measured as the volume (ml) NaOH required for a visible color change during titration. Under 1 ml NaOH is marked black, measurements over 1,50 is marked red, samples in between are marked blue to facilitate a distinction between the volumes in the table. One value is missing (14A) due to microbial spoilage of the sample.

STARTER CULTURE	17	10	3	14	12	16	11
A	1,14	1,37	1,72	-	1,61	1,88	1,72
B	1,07	1,42	1,81	1,24	1,25	1,79	1,62
C	1,39	1,71	1,74	1,06	1,60	2,06	1,75
D	1,35	1,57	1,96	1,14	1,29	1,96	1,69
E	1,08	1,47	1,68	1,31	1,25	1,46	1,55
F	1,26	1,71	1,69	1,24	1,37	1,61	1,67
G	1,61	1,61	1,84	1,34	1,69	1,69	1,72
H	0,66	1,22	1,45	0,91	1,07	1,25	1,28
I	0,95	1,52	1,75	1,09	1,20	1,71	1,47
J	1,42	1,54	1,68	1,20	1,57	1,83	1,60
K	1,22	1,37	1,64	1,26	1,39	1,40	1,55
L	1,01	1,32	1,44	0,97	1,58	1,39	1,50
M	0,99	1,24	1,51	0,83	1,17	1,23	1,30
N	0,84	1,30	1,27	0,87	1,07	1,26	1,40
O	0,79	1,29	1,35	0,92	1,28	1,25	1,30

3.2.3. Sensory characterization of yoghurts

The descriptive words generated by the panel from the initial screening of the yoghurts made with seven different proteins and 15 different starter cultures may be seen in Table 6. The yoghurts containing protein 12 were considered to taste bitter and rancid or with an off-flavour (old was the word used by the panel) to such an extent that the panel aborted the tasting session, thus only tasting the three first samples. The samples with protein 12 was rated as 1 (data not shown) and regarded as not suitable for yoghurt production. No further analyses with protein 12 were carried out.

Protein 14 gave rise to samples described as lacking in acidity, not fresh, sweet or with a good acidity but with the taste of protein 14 and not fresh. In general, the consistency of the yoghurt with protein 14 was very thick and smooth, as would be desirable for a yoghurt. All yoghurts made with protein 14 were rated as 1 (data not shown) but exhibited a very good consistency. Four samples were microbial spoiled and thus not measured

Yoghurts with protein 3, 10, 11, 16 and 17 fermented with starter cultures A, B, C and D were all described as high in acidity. Some of the combinations were also described as mild flavoured. A flavour or an acidity reminding the panel of rhubarb

was detected in yoghurts made with protein 17 and starter cultures C, D, E, F and K and in yoghurt made with some of the proteins deriving from pulses in combination with starter cultures B, C, D, E and K. Yoghurt with starter culture G was described with acidity ranging from low to high and with off-flavour, yeasty flavour or mouldy flavour for protein 17 and across all proteins deriving from pulses (except for one). Both yoghurts with starter cultures H and I were described as high in sweetness and low in acidity and off-flavour/not fresh was mentioned for starter culture I with protein 10, 3 and 11. Yoghurt with starter culture J was described as mild only with protein 17 and as acidic with the proteins deriving from pulses. Except for protein 11, a slimy texture of the yogurts fermented with starter culture J was also detected. Yoghurts with starter cultures K, L, M, N and O were all described as sweet and lacking in acidity. These yoghurts were also described as slimy or jelly (except in yoghurts with protein 17).

Based on this initial screening, together with the results of pH and TTA values, starter cultures A, B, C, D, E, G, H, I were chosen for further analysis, together with the addition of two new starter cultures; P and Q.

Table 6. Summary of the descriptive words generated from the sensory characterization of the yoghurt during the initial screening.

CULTURE	17	10	14	3	12	16	11
A	mild, medium acidity, mild oat taste, creamy, balanced, low sweetness	mild, mild flavour of pulse, low acidity	Creamy, off-flavour, no acidity	high acidity, unbalanced	off-flavour	medium acidity, salty, taste of yoghurt	medium acidity, spicy, pulse flavour
B	mild, medium acidity	mild flavour of pulse, low acidity	high acidity, taste of the protein	mild, medium acidity	off-flavour	high acidity, neutral taste, taste of rhubarb	low acidity, taste of pulse, low sweetness
C	high acidity, rhubarb flavour	medium acidity, rhubarb flavour	thick mouthfeel, high acidity, off-flavour	mild, medium acidity	off-flavour	medium acidity, thin mouthfeel	medium acidity, fruity, mild, pulse flavour
D	high acidity, rhubarb flavour	medium acidity, rhubarb flavour	thick mouthfeel, high acidity, off-flavour	high acidity, unbalanced, off-flavour, taste of yoghurt	-	medium acidity, off-flavour, thin mouthfeel	high acidity, unbalanced, neutral taste
E	low acidity, rhubarb flavor, not creamy, mild	mild, neutral taste, creamy	medium acidity, rhubarb flavour	mild, medium acidity, neutral taste, balanced	-	mild, sweet, neutral taste	medium acidity, mild, balanced, taste of rhubarb, taste of yoghurt
F	medium acidity, apple flavour, rhubarb flavour, creamy	creamy, low acidity	medium acidity, taste of the protein	mild, neutral taste, low acidity	-	medium acidity, balanced, mild, neutral	low acidity, ropy consistency, thick mouthfeel, off-flavour
G	high acidity, not creamy, off-flavour	off-flavour, sweet, low acidity	medium acidity, sweet, taste of the protein	neutral taste, medium acidity	-	medium acidity, off-flavour	low acidity, off-flavour
H	very sweet, no acidity, off-flavour, mild taste of oats, fat mouthfeeling	low acidity, off-flavour	sweet, off-flavour	neutral taste, sweet, low acidity, taste of protein	-	very sweet, lack acidity	low acidity, thin mouthfeel, low sweetness
I	Creamy, sweet, low acidity, yoghurt flavour	low acidity, off-flavour, taste of pulse	sweet, off-flavour	off-flavour	-	low acidity, thick mouthfeel, taste of pulse	Sweet, off-flavour, low acidity
J	mild, creamy, neutral taste, ropy consistency	low acidity, off-flavour	-	medium acidity, ropy consistency	-	thick mouthfeel, ropy consistency, medium acidity	high acidity, thick mouthfeel
K	rhubarb flavour, unbalanced, off-flavour, sweet, low acidity	low acidity, sweet, off-flavour	-	Sweet, medium acidity, creamy, taste of rhubarb	-	mild, balanced, sweet, low acidity, rhubarb flavour	sweet, taste of rhubarb
L	low acidity, off-flavour, thick mouthfeel, ropy consistency	mild, sweet, low acidity	-	low acidity, ropy consistency, off-flavour, neutral taste	-	sweet, low acidity	sweet, ropy consistency, taste of pulse, thick mouthfeel
M	very neutral taste, low acidity, sweet	very sweet, mild, low acidity, ropy consistency	-	mild, neutral taste, low acidity, ropy consistency	-	sweet, neutral taste, low acidity, thick mouthfeel	sweet, ropy consistency, low acidity, taste of pulse
N	very neutral, sweet, low acidity	very sweet, mild, low acidity, taste of pulse	sweet, off-flavour	mild, thick mouthfeel, low acidity	-	low acidity, thin mouthfeel	thin mouthfeel, salty, low acidity
O	off-flavour, sweet, low acidity	very sweet, mild, low acidity	sweet, no acidity	low acidity, creamy, ropy consistency	-	sweet, low acidity	sweet, low acidity, taste of pulse

3.3. Determination of fermentation capacity

3.3.1. Fermentation capacity with different proteins and starter cultures

From the initial screening of pH, TTA and sensory characteristics of the yoghurts, protein 3, 10, 11, 14, 16 and 17 in combination with starter cultures A, B, C, D, E, F, G, H, I, P and Q were selected for in-line pH measurements. In-line pH measurement was not available during the initial screening and hence not used in the beginning of this study.

Starter cultures J, K, L, M, N, O and partly F was not selected for the in-line pH measurement. Starter culture K was out of stock. Starter culture M, N, O yielded similar results in pH, TTA and initial sensory characterization and was thus omitted since starter culture H (containing the same bacteria species and yielded slightly lower pH, higher TTA and better sensory characteristics) was included. Starter culture J was omitted since it was considered to give a too ropy consistency for yoghurt application. The same applied for starter culture F in combination with protein 11 and 14.

A large variation was observed for the pH decrease over time between the starter cultures and between a specific starter culture in combination with the different proteins. Because of the large sample set, numerous fermentation curves have been generated. To exemplify the results a few of these are presented in the Appendix.

In general, the fermentation curves of the different proteins with starter cultures B (see Figure 9, Appendix), P and Q was the most similar ones which indicates that these starter cultures was less affected, compared to the other starter cultures, by the protein used in the yoghurt mixture. As seen in Table 4, protein 14 started with an initial lower pH, which was seen with all starter cultures. Yoghurts fermented with starter cultures A, C and G exhibited generally longer lag phases compared to yoghurts fermented with starter cultures B, D, E, F, H, I, P and Q, but the lag phase was very dependent on the protein included in the yoghurt. The lag phase was longer in yoghurt with proteins 10, 11, 14 and 16 (2-3 h) compared to yoghurts with protein 3 and 17 (1-1,5 h) fermented with starter culture H. Similar results were observed for starter culture I. Starter culture C in combination with protein 17 exhibited a lag phase of 40 minutes compared to protein 14 which exhibited a lag phase of approximately 5 h (as seen in Figure 10 in Appendix).

There is also a large variability in time needed to reach pH 4,3. Figure 11 (Appendix) shows that starter culture H in combination with protein 17 takes approximately 7 h to reach pH 4,3 compared to the same starter culture in combination with protein 11 which needs approximately 12,5 h. Even more contrast is seen when yoghurt with protein 10 fermented with starter culture A

(approximately 15 h) is compared to the same protein fermented with starter culture F (approximately 5,5 h) as may be seen in Figure 12 (Appendix).

Yoghurts with protein 17 seem to be least affected by which starter culture it is fermented with (see Figure 13, Appendix). The variability between the curves of the different starter cultures is not as big as for example yoghurts with protein 10 (Figure 14, Appendix) and 16 which exhibits large variations between the starter cultures ability to ferment the yoghurt with these proteins. Yoghurts with protein 10 fermented with starter culture F was the only combination that reached a pH below 4,3 in less than 6 h. Yoghurts with protein 10 and yoghurts with protein 11, was also the yoghurts that generally required the longest time to reach pH 4,3 (8,5 h-15 h) and in some cases did not reach pH 4,3 at all during the 16 hours fermentation time frame. Yoghurts with protein 3 and 16 required between 6,5-14 h to reach pH 4,3 in comparison to yoghurts with protein 14 and 17 which required between 6,5-11,5h to reach the same pH.

In general, the max acidification rate was found to be around -0,01-0,02 pH/min but for yoghurts with protein 14 it was below -0,01 pH/min for all starter cultures.

3.3.2. Fermentation capacity with different energy sources

Based on pH, TTA values, sensory characterization and in-line pH measurement, it was hypothesized that some starter cultures may not be able to ferment sucrose (which was the main sugar used in the production of the yoghurts) as well as the other starter cultures. These were the starter cultures of the samples measured as high in pH, low in TTA as well as described as very sweet/lacking in acidity and some of which pH-curves from the iCinac exhibited a slower pH decrease over time or an increase in pH in the end of the fermentation (B, H, K, L, M, N, O, P and Q).

This hypothesis was investigated by inoculating pasteurized, non homogenized oat base including different sugars. Fermentation curves for all starter cultures in combination with three energy sources; sucrose, glucose or maltose, were obtained from the iCinac software. As seen in Figure 16 (Appendix) the fermentation curves for starter culture H did not ferment maltose as well as sucrose and glucose. Similar curves were obtained for starter cultures B, M, N, O, P (data not shown). Starter culture H, M, N, O which contains *S. thermofilus* and *L. bulgaricus* do indeed not ferment maltose. Starter cultures B and P contains LAB which in general should be able to ferment maltose but seem to contain a strain which struggles a bit with maltose anyway (se Figure 17, Appendix).

For all starter cultures, fermentation with maltose did not yield as low final pH as fermentation with sucrose and glucose did. Fermentation curves for sucrose and glucose were fairly similar in most cases.

3.4. Growth of LAB

To confirm an actual growth of the starter cultures during fermentation, the live count of the bacteria was determined before and after fermentation. The plating of starter cultures on two different agar selective for Lactobacillus and Streptococci, confirmed the growth of LAB for both genus. Figure 1 shows the growth of Lactobacilli before and after fermentation in the yoghurts with different proteins.

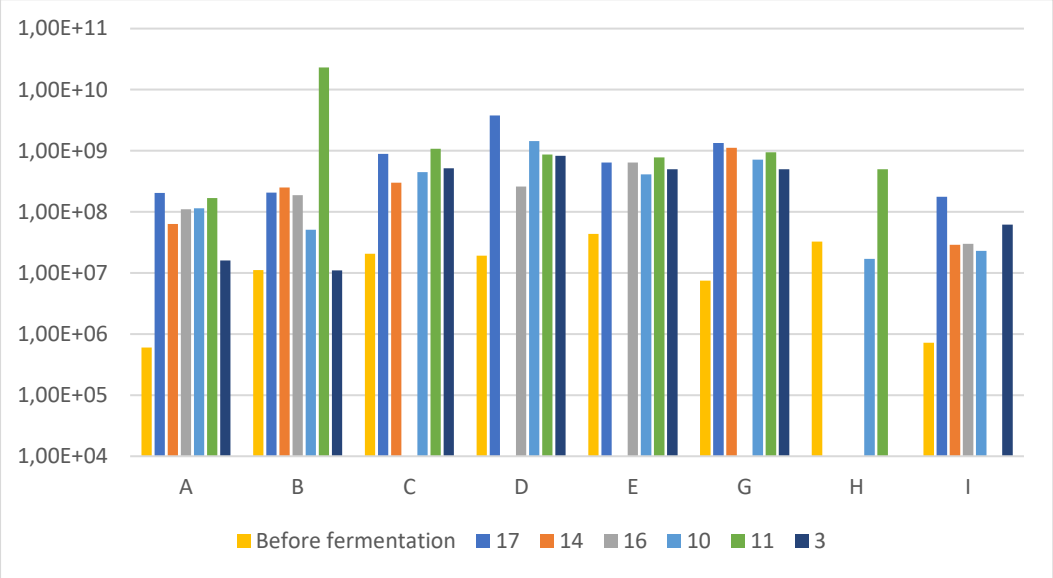

Figure 1. Growth of Lactobacillus in the starter cultures during fermentation with different proteins. Missing data for some proteins are due to plates which could not be counted.

Plating of *S. thermophilus* also confirmed a growth of bacteria during fermentation, as may be seen in Figure 2.

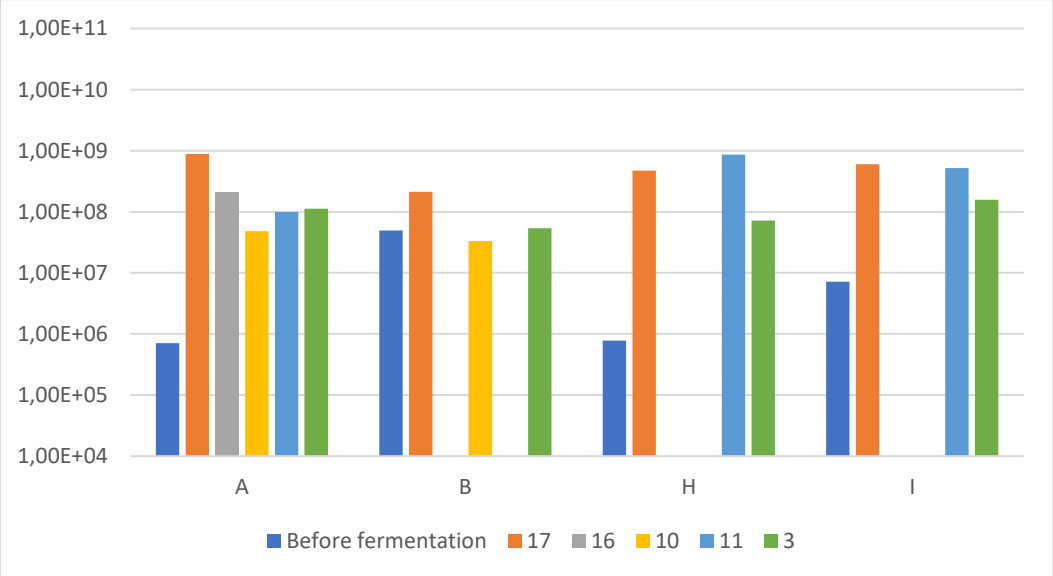

Figure 2. Growth of S. thermophilus in the starter cultures during fermentation with different proteins. Missing data for some proteins are due to plates which could not be counted

3.5. Sensory profiling

Based on the results from the initial pH measurements, TTA values and sensory screening of yoghurts, as well as the in-line pH measurements with different carbon sources, starter cultures A, B, C, D, E, G, H, I were chosen for further analysis. They were estimated to have potential for plant-based yoghurt application using sucrose as added sugar source, yielding a yoghurt with low pH, high TTA and good sensory attributes. The selection was also based on choosing starter cultures containing different bacteria, to keep the broad spectrum of starter cultures in this study. The previous used protein content of 3 % was perceived as taking over the sensory attributes owed to the starter cultures and was thus lowered to 2,5 %.

The intensity of attributes for the chosen starter cultures in combination with the six plant proteins is visualized in Figures 3-8 in the following sections 3.5.1.-3.5.6.

3.5.1. Protein 17

When it comes to acidity, yoghurts fermented with starter culture C, D and G were considered to be the most acidic ones (see Figure 3). These three yoghurts were also rated as the least sweet ones. Yoghurt fermented with starter culture H was the sweetest one simultaneously as being least acidic.

When it comes to creaminess, starter cultures A, H and I was considered to give rise to yoghurts with higher creaminess. Yoghurts with starter cultures C and H was considered to have the fruitiest aroma while yoghurt with culture I was the least fruity one. Yoghurts with starter cultures B and I appeared to taste the most protein while yoghurts fermented with starter cultures C, E and H was considered to have the lowest taste of protein. Starter culture G and I was considered to give rise to the most off-flavours in contrast to culture C and E which gave the lowest off-flavour among the yoghurts made with protein 17.

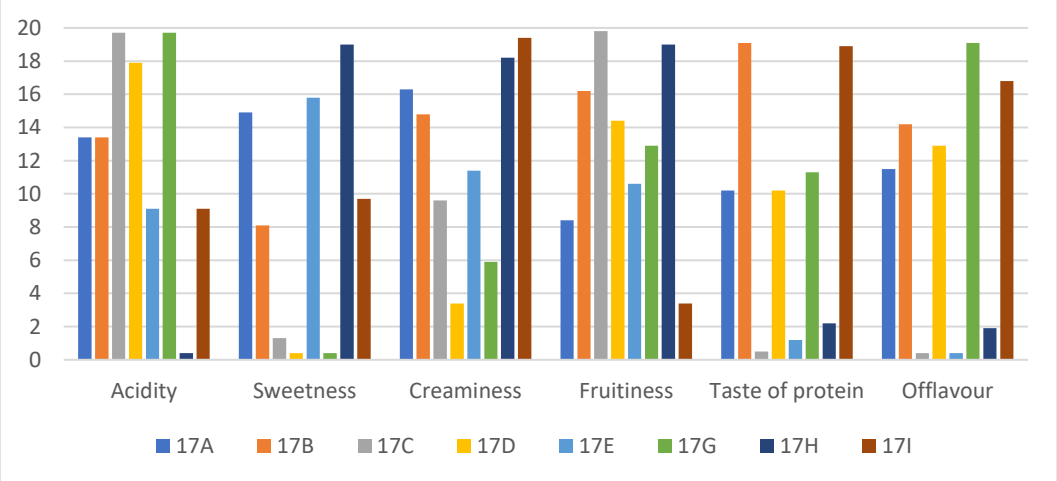

Figure 3. Sensory profile of yoghurts with protein 17 fermented with starter cultures A, B, C, D, E, G, H, and I.

3.5.2. Protein 14

As seen in Figure 4 the most acidic yoghurt made with protein 14 was fermented with starter culture B, followed by culture H, A and I. Yoghurts fermented with starter cultures C, E and D were all rated as lowest in acidity. When it comes to sweetness, yoghurts fermented with starter cultures C and D were percieved as most sweet, followed by E and G. The yoghurt fermented with starter culture B was assessed as least sweet. Regarding creaminess of the yoghurts, starter culture D was percieved as giving the creamiest yoghurt followed by starter culture H and I. Least creamy was yoghurts fermented with starter culture A and B. Yoghurt fermented with starter culture H was percieved as the most friuty one, followed by starter culture I and B. Starter culture C and E gave the least fruity yoghurts. Most taste of protein was found in yoghurts fermented with starter culture C, followed by I and E. Starterr culture G and H gave the least taste of protein in the yoghurt. Most off-flavours were found in yoghurt fermented with starter culture E and C in contrast to starter culture I and B where least off-flavours were found.

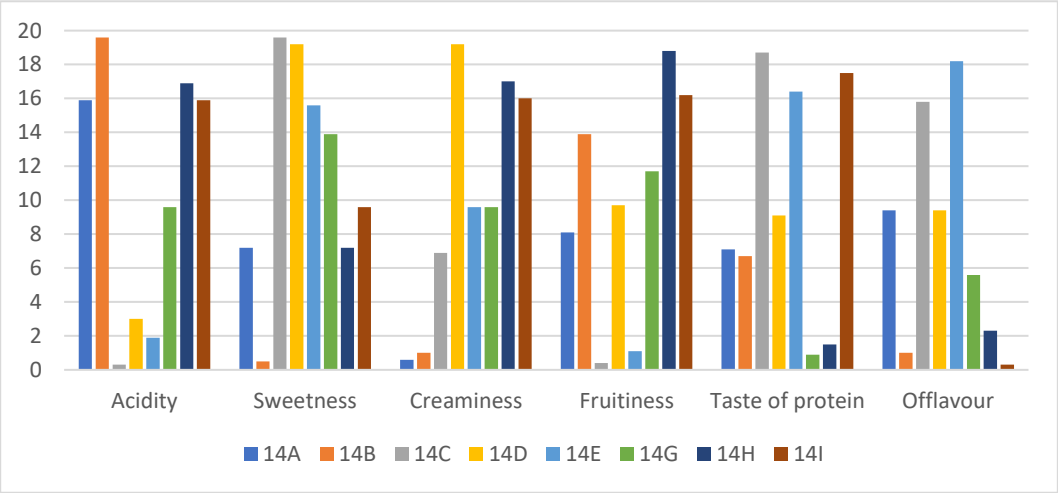

Figure 4. Sensory profile of yoghurts with protein 14 fermented with starter culture A, B, C, D, E, G, H, and I.

3.5.3. Protein 16

In yoghurts made with protein 16 the highest acidity was found when fermented with starter culture E and C, as seen in Figure 5. Lowest acidity was found in yoghurt fermented with starter culture H followed by starter culture B and I. Highest sweetness was found in yoghurt fermented with starter culture H, A and B in contrast to lowest sweetness which was found with starter culture E. Starter culture I and G were perceived as giving the most creamy yoghurts in contrast to A, E and C which were found to give the least creamy yoghurts. Most fruitiness was found in yoghurt fermented with starter culture C, followed by E. Least fruitiness was found in yoghurts fermented with starter culture H and I. Most taste of protein was found in yoghurt with starter culture I and G compared to the lowest taste of protein

found with starter culture H. Most off-flavours were perceived in the yoghurts made with starter culture G and I while yoghurts fermented with starter cultures H, C and A were perceived as tasting the least off-flavours.

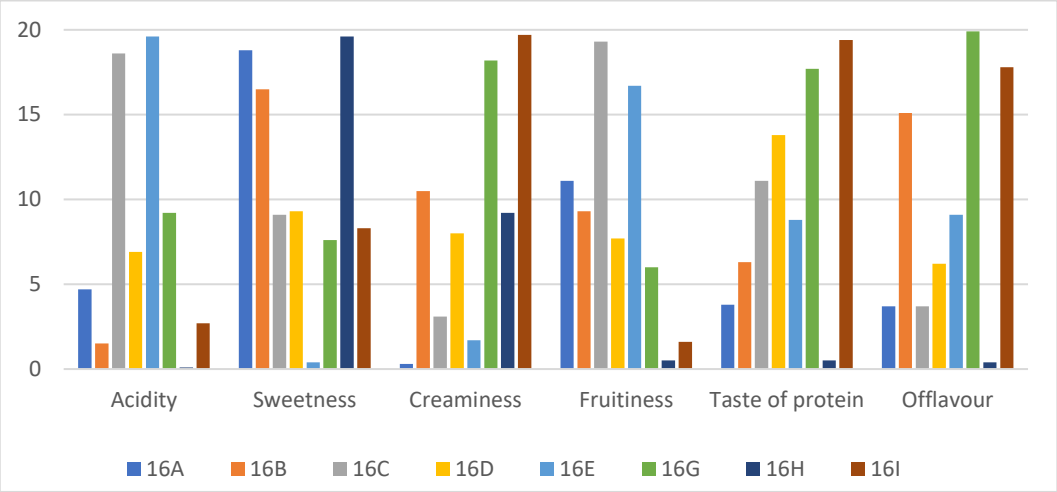

Figure 5. Sensory profile of yoghurt with protein 16 fermented with starter culture A, B, C, D, E, G, H, and I.

3.5.4. Protein 10

Protein 10 together with starter culture G was perceived as the yoghurt with highest acidity, followed by yoghurts fermented with C, A and D, as may be seen in Figure 6. Yoghurts fermented with starter culture I and H were rated as lowest in acidity. Yoghurt with starter culture H was also rated as highest in sweetness and yoghurts fermented with starter cultures G and C as lowest in sweetness. Regarding creaminess, starter culture I gave rise to the creamiest yoghurt, followed by A and E. In comparison, starter culture G was perceived as giving rise to the least creamy yoghurt. Starter cultures C and E were perceived as producing the fruitiest yoghurts while yoghurts with starter cultures G, H and I were perceived as the least fruity ones. Highest taste of protein was ascribed to yoghurt fermented with starter culture C, followed by A and B. Lowest taste of protein was found in yoghurts fermented with cultures G and D. In contrast, the highest off-flavour was found in yoghurts fermented with starter cultures G and H and the lowest off-flavour was found in yoghurt fermented with starter culture E and D.

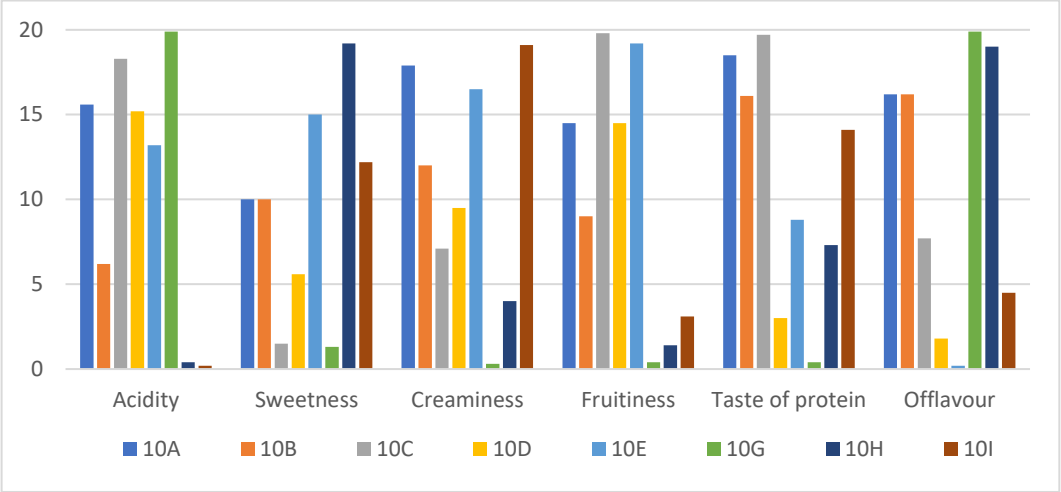

Figure 6. Sensory profile of yoghurt with protein 10 fermented with starter culture A, B, C, D, E, G, H, and I.

3.5.5. Protein 11

As seen in Figure 7 the highest acidity in yoghurts with protein 11 was reached when fermented with starter culture G, followed by starter culture C and D. Yoghurts fermented with starter culture I and H was rated as lowest in acidity. Highest sweetness was found in yoghurts fermented with starter cultures I, H and B and lowest sweetness was found in yoghurts fermented with starter cultures C, D and G. Creaminess was perceived as highest in yoghurt fermented with starter culture H, followed by I and D. Lowest creaminess was found in yoghurts fermented with starter cultures C and G. Yoghurts fermented with starter culture C was rated as the highest regarding fruitiness and starter cultures I and H as lowest in fruitiness. The taste of protein was determined to be highest in yoghurt fermented with starter culture A, followed by starter cultures B and C. The taste of protein was perceived as lowest in yoghurt fermented with starter culture H. The taste of off-flavours was highest in yoghurt fermented with starter culture G, followed by C and A. The taste of off-flavours was lowest in yoghurts fermented with I and H.

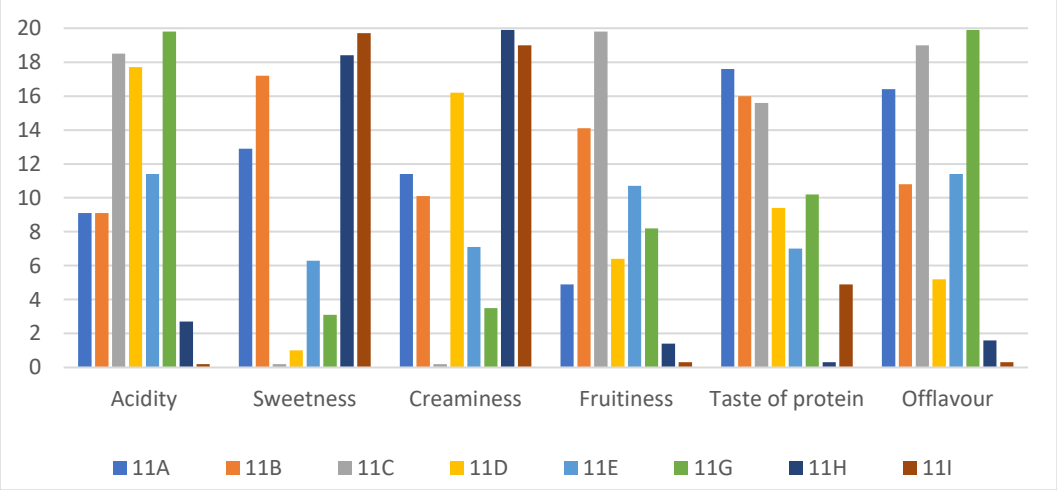

Figure 7. Sensory profile of yoghurts with protein 11 fermented with starter culture A, B, C, D, E, G, H, and I.

3.5.6. Protein 3

In yoghurts with protein 3, fermentation with starter culture G yielded the highest acidity, followed by fermentation with starter culture C (seen in Figure 8). Lowest acidity was ascribed to yoghurts fermented with H and I. In contrast, yoghurts fermented with starter cultures H and I was described as highest in sweetness as well as highest in creaminess. Lowest sweetness was ascribed to yoghurts fermented with starter cultures G, E and C. Yoghurt fermented with starter culture G was also described as the one with lowest creaminess. Highest fruitiness was detected in yoghurt fermented with starter culture C, followed by D and E. Lowest fruitiness was ascribed to yoghurts fermented with starter cultures I, H and G. Fermentation with starter culture G gave rise to the yoghurt with highest taste of protein, followed by yoghurts fermented with starter cultures A, B and C. Lowest taste of protein was found in yoghurts fermented with starter culture I. The highest off-flavour was found in yoghurt fermented with starter culture C, followed by G, while the lowest off-flavour was found in yoghurts fermented with starter culture E, I and B.

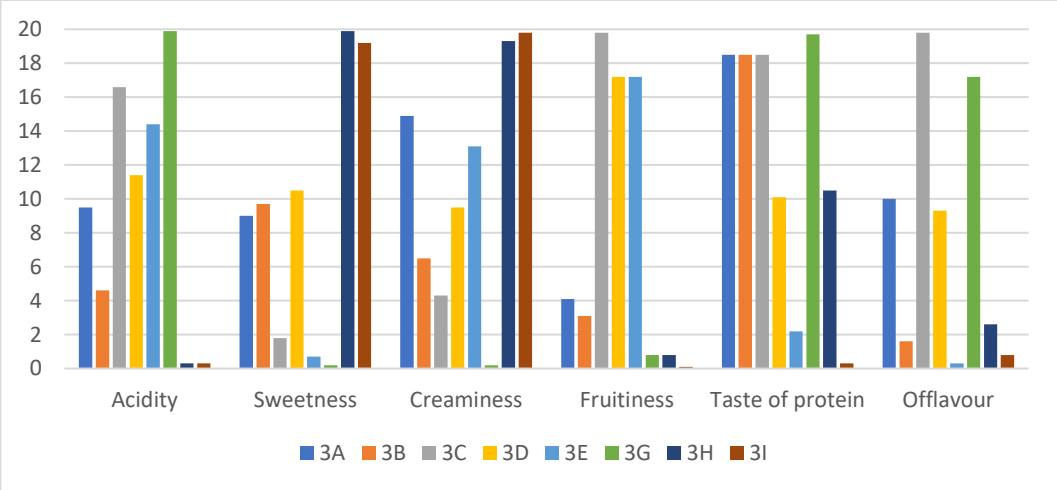

Figure 8. Sensory profile of yoghurts with protein 3 fermented with starter culture A, B, C, D, E, G, H, and I.

3.5.7. Conclusion of sensory profiling

In conclusion, each sensory profile is unique for the starter culture in combination with the specific protein. In Table 7 the highest scored combinations of starter culture and proteins for each attribute is summarized. In comparison, the lowest scored combinations of starter culture and protein for each attribute is summarized in Table 8. Even though the sensory profiles between each protein are not comparable there are some conclusions that may be drawn.

Most yoghurts fermented with starter culture G was rated to yield the highest acidity. Yoghurts fermented with starter culture H seemed to give rise to the highest sweetness in most yoghurts, while yoghurts fermented with starter culture I seemed

to develop the highest creaminess. Yoghurts fermented with starter culture C was assessed most times as being the fruitiest yoghurts. Highest taste of protein seemed more difficult to ascribe to one starter culture but yoghurt fermented with starter culture A was rated as highest twice. Yoghurts fermented with starter culture G was rated most times as having the most off-flavours.

Table 7. Highest rated combinations of starter culture and proteins of each attribute from the sensory profiles

Highest rated yoghurt for each attribute						
	17	**14**	**16**	**10**	**11**	**3**
Acidity	C/G	B	E	G	G	G
Sweetness	H	C	H	H	I	H
Creaminess	I	D	I	A	H	I
Fruitiness	C	H	C	C	C	C
Taste of protein	B	C	I	A	A	G
Off-flavour	G	E	G	G	G	C

Lowest acidity was most times found in yoghurts fermented with starter cultures I and H. Starter culture G seemed to develop the lowest sweetness in most yoghurts while it was also rated, as well as starter culture A, as yielding the lowest creaminess in most yoghurts. Lowest fruitiness may be ascribed to starter culture I in most cases. Lowest taste of protein as well as off-flavour seemed more difficult to ascribe to one starter culture, but starter culture G and H was rated equal times as yielding yoghurts with the lowest taste of proteins while starter culture E and I was rated most times as producing yoghurts with the lowest off-flavours.

Table 8. Lowest rated combinations of starter culture and proteins of each attribute from the sensory profiles

Lowest rated yoghurts for each attribute						
	17	**14**	**16**	**10**	**11**	**3**
Acidity	H	C	H	I	I	H/I
Swetness	G	B	E	G	C	G
Creaminess	G	A	A	G	C	G
Fruitiness	I	C	H	G	I	I
Taste of protein	E	G	H	G	H	I
Off-flavour	E	I	H	E	I	E

4. Discussion

The conducted screening of starter cultures for oat-based non-dairy yoghurt demonstrates the broad spectrum of possible variations of plant-based fermented products. It clearly shows the importance to choose specific starter cultures suited for the results when developing new plant-based fermented products. The increased consumption of plant-based products depends on several reasons, such as environmental, nutritional and ethical, but in the end the product has to taste good in order for the consumer to buy it more than once.

The project was much based on the hypothesis that traditional yoghurt starter cultures *S. thermophilus* and *L. bulgaricus* may not be the most suitable starter cultures when it comes to plant-based yoghurts. The traditional starter cultures used in this screening measured a generally higher pH, lower amount of acids and were sensory described as sweet and lacking in acidity. One traditional starter culture (H) was used in the sensory profiling test and was the starter culture described as giving the sweetest yoghurts in most cases. It was also described as one out of two starter cultures that yielded the lowest taste of protein.

Most of the measured pH values of the yoghurts were below pH 4,3. The yoghurts with pH above 4,3 were most centralized to yoghurts with protein 14 and 16 and to yoghurts fermented with the starter cultures containing the traditional LAB for dairy yoghurt (starter culture H, M, N, O). Previous studies have reported pH ranging from 3,9-4,5 after 16 h of oat fermentation (*L. plantarum*, *L. del. bulgaricus*, *S. thermophilus*, *L. acidophilus* and *Bifidobacterium* spp.) (Russo *et al.*, 2016; Mårtensson *et al.*, 2001). A final pH below 4,3 could be seen as an indication of good growth conditions in fermented oat milk (Mårtensson *et al.*, 2002; Mårtensson *et al.*, 2001). The high pH of the yoghurt made with protein 12 indicates that protein 12 is not the most suitable protein used in this fermentation matrix, with the chosen starter cultures.

Compared to other plant based non-dairy yoghurts on the market, the measurement of TTA was equivalent to other brands. However, no yoghurts fermented in this screening exhibited an amount of TTA comparable to that of a dairy yoghurt.

The iCinac method, used to measure the pH value in-line during the fermentation, is a well-established method within the food fermentation industry. Variations in pH decrease over time within the same protein, starter culture or

energy source most probably depends on the complexity of the food system which includes live bacteria, fluctuating amounts of sugar and dry matter of the oat base and small variations of temperature.

The in-line pH fermentation with different energy sources revealed an increase in pH for some starter cultures. The increase in pH may be explained by the particular starter cultures inability to ferment maltose, thus seeking its energy from amino acids instead. This leads to a release of ammonium ions which causes the increase in pH. Yoghurts which were perceived as sweet may contain starter cultures which do not ferment maltose which is the primary sugar in the oat base. The maltose is thus left in the oat base and gives rise to a sweet yoghurt. This was probably the case for the starter cultures which exhibited a poor fermentation with the maltose and were used in the fermentation with yoghurts described as sweet during the initial sensory screening. Some fermentation curves exhibited a very sharp rise in pH (data not shown). This may partly be explained by the inability for that particular starter culture to ferment the sugar but more reasonable this is due to instrumental errors or disturbance.

When it comes to choosing the right starter culture and protein combinations during product development the combinations of starter culture and protein should be chosen partly with consideration to the time limitations within the food industry. Normally a dairy yoghurt is fermented during 4 hours (Adams & Moss, 2008). Lag phase, Max acidification rate as well as Time at max acidification rate are all important aspects when it comes to this time limitation. In this project, only one starter culture reached pH 4,3 under 6 hours. There are many factors that could have been altered to try to decrease this time. Temperature at which fermentation takes place is one important factor that could influence this time frame but was not included to be investigate in this project.

The texture of the yoghurts was not either measured during this screening since the limited amount of sample and time did not support any more measurements. The texture would however have been another interesting feature to investigate since different textures owed to the starter cultures were noticed during this project. It was however observed that the viscosity of the yoghurts was similar to a dairy yoghurt and that some starter cultures gave rise to more ropy textures than others.

The live counts confirmed a growth with 1-2 log units, which is to be expected in fermentation of a dairy yoghurt (Adams & Moss, 2008). The samples varied a lot, and some were omitted since some of the plates could not be interpreted. To verify these results more repetitions must be carried out.

During the initial screening, the yoghurts were assessed by unstructured discussions about each yoghurt. This was used as a fast method of yielding a lot of attributes simultaneously as exposing the panel for a large set of different samples. The large list of generated attributes was reduced into a shorter list of descriptive words later used for the sensory profile test. By default, this resulted in fewer

nuances between the descriptions of the yoghurts, losing some of the descriptive words and omitting hedonic and texture describing words.

Because of an unfamiliarity to the method the panel found it difficult to assess astringency and bitterness in the initial sensory characterization. They also had difficulties in finding the correct descriptive words and it was not clear if the description not fresh was related to a lack in acidity or off-flavours detected by the panel members.

The sensory profiling method was chosen because the sensory profile of each yoghurt was a good way to differentiate the starter cultures in combination with different proteins. The carried out sensory profiling was based on traditional sensory profiling techniques (Lawless & Heymann, 2010). Due to limited time and resources within the food industry the extensive training of the panel had to be omitted and was replaced with a consensus discussion at the end of each session. The consensus discussion was used as the result for each sensory profile. However, the initial sensory screening process was constructed to have a dual purpose; to carry out the initial sensory screening and to train the panel to assess the yoghurts prior to the profile test. Due to Covid19 the assessors were not exactly the same in the initial sensory characterization as they were in the sensory profiling tests (nor the same between the sessions of the profiling tests), which contributed to minimized accuracy of the profiling tests.

The eight yoghurts assessed in each session took 1 h to evaluate and was experienced as the maximum amount of samples which the panel could asses per session, but six samples would have been the preferred maximum amount of samples to increase the accuracy of the assessment (Gustafsson *et al.*, 2014). Even though the descriptive words used in the sensory profile test were in line with previous used attributes for sensory evaluation of yoghurts, the panel experienced difficulties in differentiating between off-flavour and taste of protein respective acidity, sweetness and fruitiness, since the thought of fruit correlated with both acidity and sweetness (Yuceer & Drake, 2007). More extensive training with reference samples of different attributes, better defined attributes and screening the panel for thresholds of the five basic tastes could have minimized errors during the sensory profiling.

Further sensory tests of individual starter cultures in combination with a specific protein within a specific food matrix, could focus on carrying out consumer acceptance and preference tests. Using the JAR-scale in which the assessors decides if specific attributes of the sample tastes too much, too little or just about right for a new product would be useful to use since it combines intensity and hedonic assessment (Lawless & Heymann, 2010).

As Table 7 and 8 summarizes, yoghurts fermented with starter culture G seemed to yield the highest acidity in combination with most proteins and consequently was also described most times as yielding the lowest sweetness, which is in line with

the fact that tartness of acidity may be reduced by sugars (Sadler & Murphy, 2010). If one is looking for an acidic final product, starter culture G in combination with protein 17, 10, 11 or 3 could be used. If one instead wants a final product with a high sweetness one could choose starter culture H in combination with protein 17, 16, 10 or 3.

This screening gave rise to a large set of data and the results may be seen upon as indications. No replicates, repetitions or statistical analysis was applied in this project since it aimed to be a screening covering a large sample set in a limited time. However, the numerous in-line pH measurements carried out for each starter culture but with varying proteins showed trends for each starter culture supporting the reported results. The sensory profiles of the yoghurts also showed trends for each starter culture even though the proteins in the yoghurt varied between each session.

The results from this report may be used as a database of different starter cultures in combination with different plant proteins and may be further extended to include other starter cultures as well as proteins. The results can be used as a starting point in product development of plant based fermented products but further analysis and replicates should be carried out to confirm the results of the individual combinations of starter cultures and proteins within a specific food matrix.

In conclusion, each starter culture behaves differently in different food matrixes. In this screening it has been demonstrated that oat based yoghurt fermented with the traditional yoghurt starter cultures (*S. thermophilus* and *L. bulgaricus*) gave rise to higher pH, lower amount of lactic acid and appeared sweeter compared to some of the alternative starter cultures. Consequently, there are other starter cultures available on the market which exhibits better potential for plant-based yoghurt and the choice of starter culture and protein dictates the characteristics of a plant-based yoghurt.

5. References

Adams, M.R. & Moss, M.O. (2008). *Food microbiology*. Three. ed: RCS publishing.

Baran, E., Büyükkileci, A.O. & Harsa, Ş. (2012). Screening of aroma profiles for artisanal yogurt starter cultures. *New Biotechnology,* 29, p. S233.

Bintsis, T. (2018). Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. *AIMS microbiology,* 4(4), pp. 665-684.

Bruckner-Guhmann, M., Vasil'eva, E., Culetu, A., Duta, D., Sozer, N. & Drusch, S. (2019). Oat protein concentrate as alternative ingredient for non-dairy yoghurt-type product. *J Sci Food Agric,* 99(13), pp. 5852-5857.

Brückner-Gühmann, M., Banovic, M. & Drusch, S. (2019). Towards an increased plant protein intake: Rheological properties, sensory perception and consumer acceptability of lactic acid fermented, oat-based gels. *Food Hydrocolloids*, 96, pp. 201-208.

Ceapa, C., Lambert, J., van Limpt, K., Wels, M., Smokvina, T., Knol, J. & Kleerebezem, M. (2015). Correlation of Lactobacillus rhamnosus Genotypes and Carbohydrate Utilization Signatures Determined by Phenotype Profiling. *Applied and environmental microbiology,* 81(16), pp. 5458-5470.

Farnworth, E. (2008). *Handbook of fermented functional foods*. 2. ed: CRC Press.

Fritsch, C., Vogel, R.F. & Toelstede, S. (2015). Fermentation performance of lactic acid bacteria in different lupin substrates - influence and degradation ability of antinutritives and secondary plant metabolites. *Journal of applied microbiology*(119), pp. 1075-1088.

González-Pérez, S. & Arellano, J.B. (2009). 15 - Vegetable protein isolates. In: Phillips, G.O. & Williams, P.A. (eds) *Handbook of Hydrocolloids (Second Edition)* Woodhead Publishing, pp. 383-419. Available from: http://www.sciencedirect.com/science/article/pii/B9781845694142500151.

Gu, Y., Li, X., Liu, H., Li, Q., Xiao, R., Dudu, O.E., Yang, L. & Ma, Y. (2020). The impact of multiple-species starters on the peptide profiles of yoghurts. *International Dairy Journal,* 106, p. 104684.

Gustafsson, I.-B., Jonsäll, A., Mossberg, L., Swahn, J. & Öström, Å. (2014). *Sensorik och marknadsföring*. Lund: Studentlitteratur.

Hammes, W.P. & Vogel, R.F. (1995). The genus Lactobacillus. In: Wood, B.J.B. & Holzapfel, W.H. (eds) *The Genera of Lactic Acid Bacteria*. Suffolk: Chapman & Hall, pp. 19-54.

Hardie, J.M. & Whiley, R.A. (1995). The genus Streptococcus. In: Wood, B.J.B. & Holzapfel, W.H. (eds) *The genera of Lactic Acid Bacteria*. Suffolk: Chapman & Hall, pp. 53-124.

Hedberg, M., Hasslöf, P., Sjöström, I., Twetman, S. & Stecksén-Blicks, C. (2008). Sugar fermentation in probiotic bacteria : an in vitro study. *Oral Microbiology and Immunology,* 23(6), pp. 482-485.
Intelligens, E. (2006). *The European Market for Soya and Non-dairy Drinks*.
Jeske, S., Zannini, E. & Arendt, E.K. (2017). Evaluation of Physicochemical and Glycaemic Properties of Commercial Plant-Based Milk Substitutes. *Plant Foods Hum Nutr,* 72(1), pp. 26-33.
Kampen, W.H. (2014). Chapter 4 - Nutritional Requirements in Fermentation Processes. In: Vogel, H.C. & Todaro, C.M. (eds) *Fermentation and Biochemical Engineering Handbook (Third Edition)*. Boston: William Andrew Publishing, pp. 37-57. Available from: http://www.sciencedirect.com/science/article/pii/B9781455725533000040.
Lawless, H.T. & Heymann, H. (2010). *Sensory Evaluation of Food - Principles and Practices*: Springer-Verlag New York.
Leroy, F. & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology,* 15(2), pp. 67-78.
Loponen, J., Laine, P., Sontag-Strohm, T. & Salovaara, H. (2007). Behaviour of oat globulins in lactic acid fermentation of oat bran. *European Food Research and Technology,* 225(1), pp. 105-110.
Meilgaard, M.C. & Carr, B.T. (2006). *Sensory Evaluation Techniques*: CRC Press. Available from: https://books.google.se/books?id=leTKBQAAQBAJ.
Mintel (2011). In the shadow of competion, the soy market slumps. Mintel.
Mårtensson, O., Andersson, C., Andersson, K., Öste, R. & Holst, O. (2001). Formulation of an oat-based fermented product and its comparison with yoghurt. *Journal of the Science of Food and Agriculture,* 81(14), pp. 1314-1321.
Mårtensson, O., Öste, R. & Holst, O. (2000). Lactic Acid Bacteria in an Oat-based Non-dairy Milk Substitute: Fermentation Characteristics and Exopolysaccharide Formation. *LWT - Food Science and Technology,* 33(8), pp. 525-530.
Mårtensson, O., Öste, R. & Holst, O. (2002). Texture promoting capacity and EPS formation by lactic acid bacteria in three different oat-based non-dairy media. *European Food Research and Technology,* 214(3), pp. 232-236.
Mäkinen, O., Wanhalinna, V., Zannini, E. & Arendt, E. (2015). Foods for Special Dietary Needs: Non-Dairy Plant Based Milk Substitutes and Fermented Dairy Type Products. *Critical Reviews in Food Science and Nutrition*.
Mäkinen, O.E., Sozer, N., Ercili-Cura, D. & Poutanen, K. (2017). Chapter 6 - Protein From Oat: Structure, Processes, Functionality, and Nutrition. In: Nadathur, S.R., Wanasundara, J.P.D. & Scanlin, L. (eds) *Sustainable Protein Sources*. San Diego: Academic Press, pp. 105-119. Available from: http://www.sciencedirect.com/science/article/pii/B9780128027783000068.
Pokusaeva, K., Fitzgerald, G.F. & van Sinderen, D. (2011). Carbohydrate metabolism in Bifidobacteria. *Genes & nutrition,* 6(3), pp. 285-306.
Ruas-Madiedo, P., Hernández-Barranco, A., Margolles, A. & de los Reyes-Gavilán, C.G. (2005). A bile salt-resistant derivative of Bifidobacterium

animalis has an altered fermentation pattern when grown on glucose and maltose. *Applied and environmental microbiology*, 71(11), pp. 6564-6570.
Russo, P., de Chiara, M.L.V., Capozzi, V., Arena, M.P., Amodio, M.L., Rascón, A., Dueñas, M.T., López, P. & Spano, G. (2016). Lactobacillus plantarum strains for multifunctional oat-based foods. *LWT - Food Science and Technology*, 68, pp. 288-294.
Sadler, G. & Murphy, P. (2010). PH and Titratable Acidity. In: *Food analysis*, pp. 219-238.
Salmerón, I. (2014). Effect of substrate composition and inoculum on the fermentation kinetics and flavour compound profiles of potentially non-dairy probiotic formulations. *Lebensmittel-Wissenschaft + [i.e. und] Technologie*, v. 55(no. 1), pp. pp. 240-247-2014 v.55 no.1.
Salmeron, I., Fuciños, P., Charalampopoulos, D. & Pandiella, S.S. (2009). Volatile compounds produced by the probiotic strain Lactobacillus plantarum NCIMB 8826 in cereal-based substrates. *Food Chemistry*, 117(2), pp. 265-271.
Shand, P.J., Ya, H., Pietrasik, Z. & Wanasundara, P.K.J.P.D. (2007). Physicochemical and textural properties of heat-induced pea protein isolate gels. *Food Chemistry*, 102(4), pp. 1119-1130.
Simpson, W.J. & Taguchi, H. (1995). The genus Pediococcus, with notes on the genera Tetratogenococcus and Aerococcus. In: Wood, B.J.B. & Holzapfel, W.H. (eds) *The genera of Lactic Acid Bacteria*. Suffolk: Chapman & Hall, pp. 125-172.
Soomro, A.H. & Masud, T. (2008). Selection of yoghurt starter culture from indigenous isolates ofStreptococcus thermophilus andLactobacillus delbrueckii subsp.bulgaricus on the basis of technological properties. *Annals of Microbiology*, 58(1), pp. 67-71.
Sumesh, K. & Roshan, D. (2019). *Dairy Alternatives Market by Source (Soy, Almond, Rice and Other Sources), Application (Food, Beverages, Dairy-free Probiotic Drinks and Others), Distribution Channel (Large Retail, Small Retail, Specialty Stores and Online): Global Opportunity Analysis and Industry Forecast, 2019-2026*.
Tian, H., Shi, Y., Zhang, Y., Yu, H., Mu, H. & Chen, C. (2019). Screening of aroma-producing lactic acid bacteria and their application in improving the aromatic profile of yogurt. *Journal of Food Biochemistry*, 43(10), p. e12837.
Tulbek, M., Lam, R., Wang, C., Asavajaru, P. & Lam, A. (2016). Pea: A Sustainable Vegetable Protein Crop. In, pp. 145-162.
van den Bogaard, P.T., Hols, P., Kuipers, O.P., Kleerebezem, M. & de Vos, W.M. (2004). Sugar utilisation and conservation of the gal-lac gene cluster in Streptococcus thermophilus. *Syst Appl Microbiol*, 27(1), pp. 10-7.
Yuceer, Y. & Drake, M. (2007). Sensory Analysis of Yogurt. In, pp. 265-278.

Acknowledgements

Kerstin Holmgren
Hasse Jonsson
Iwona Kihlberg

6. Appendix

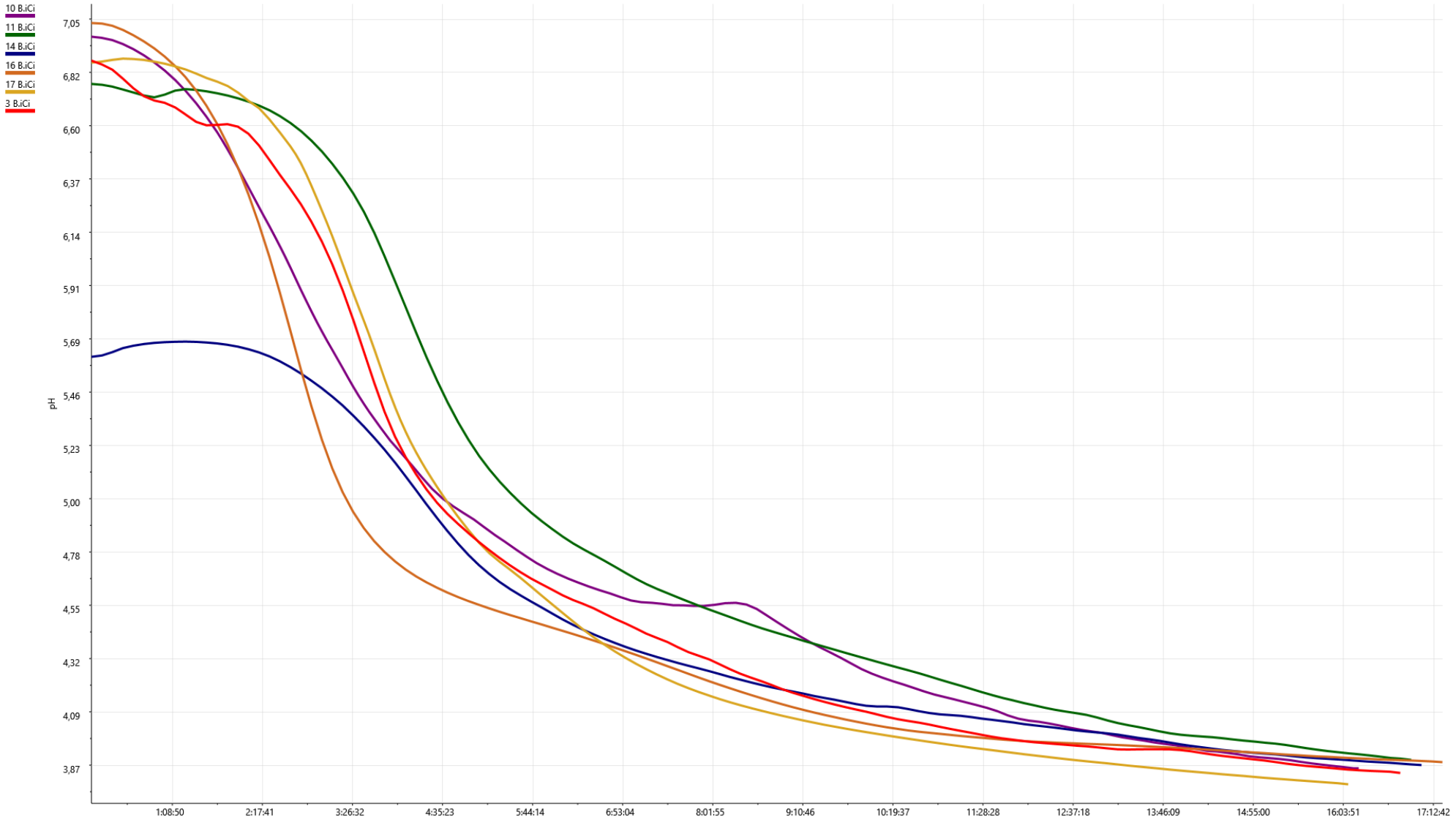

Figure 9. Fermentation curve of yoghurt fermented with starter culture B in combination with protein 10 (purple), 11 (green), 14 (blue), 16 (orange), 17 (yellow) and 3 (red).

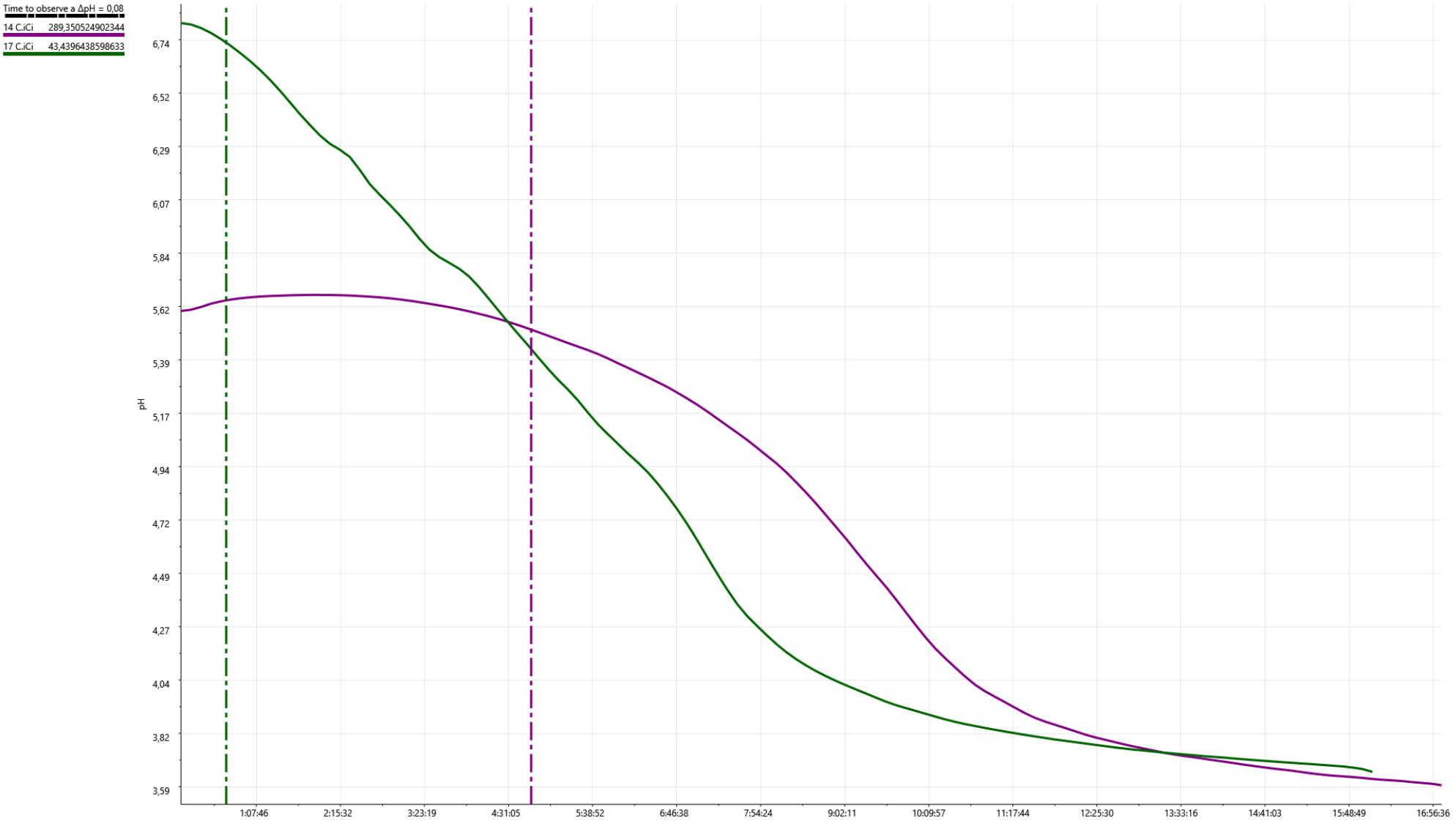

Figure 10. Fermentation curves of yoghurts fermented with starter culture C in combination with protein 14 (purple) and 17 (green). The standard lag phase is market in the graph for each curve.

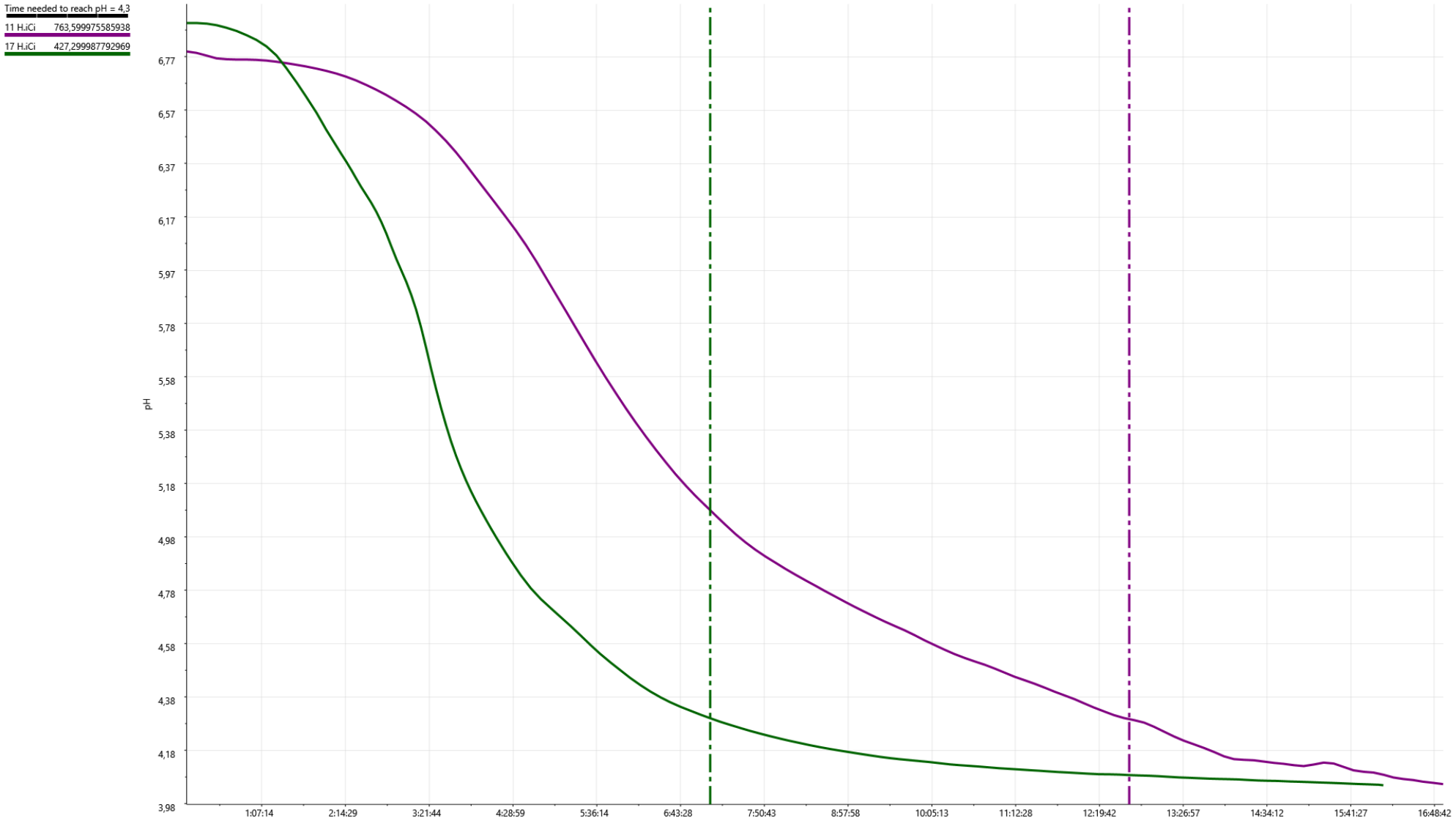

Figure 11. Fermentation curves of yoghurt fermented with starter culture H in combination with protein 11 (purple) and 17 (green). The time needed to reach a pH of 4,3 is market in the graph for each curve.

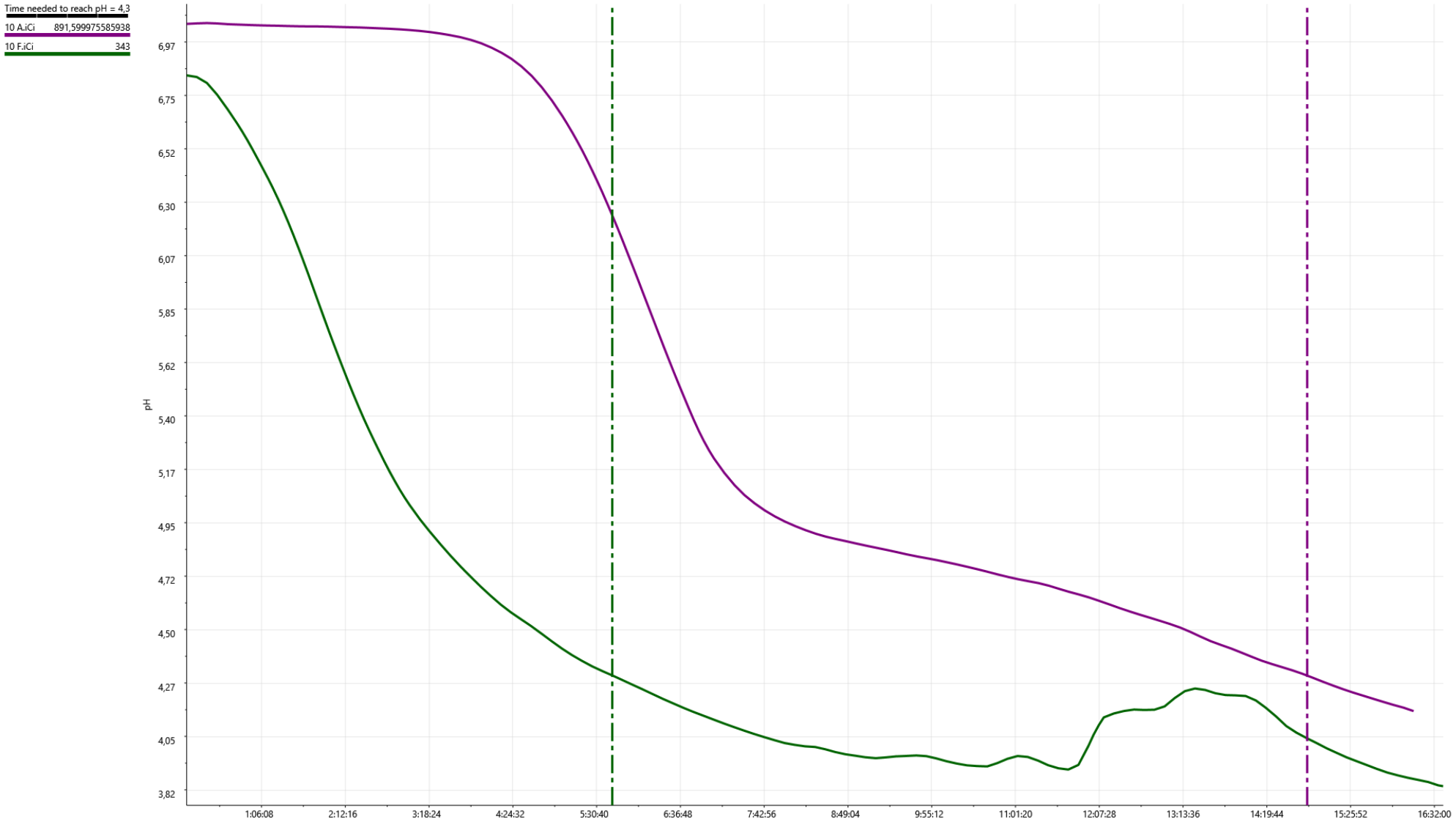

Figure 12. Fermentation curve of yoghurt fermented with starter culture A (purple) and F (green) in combination with protein 10. The time needed to reach pH 4,3 is marked in the graph for each curve.

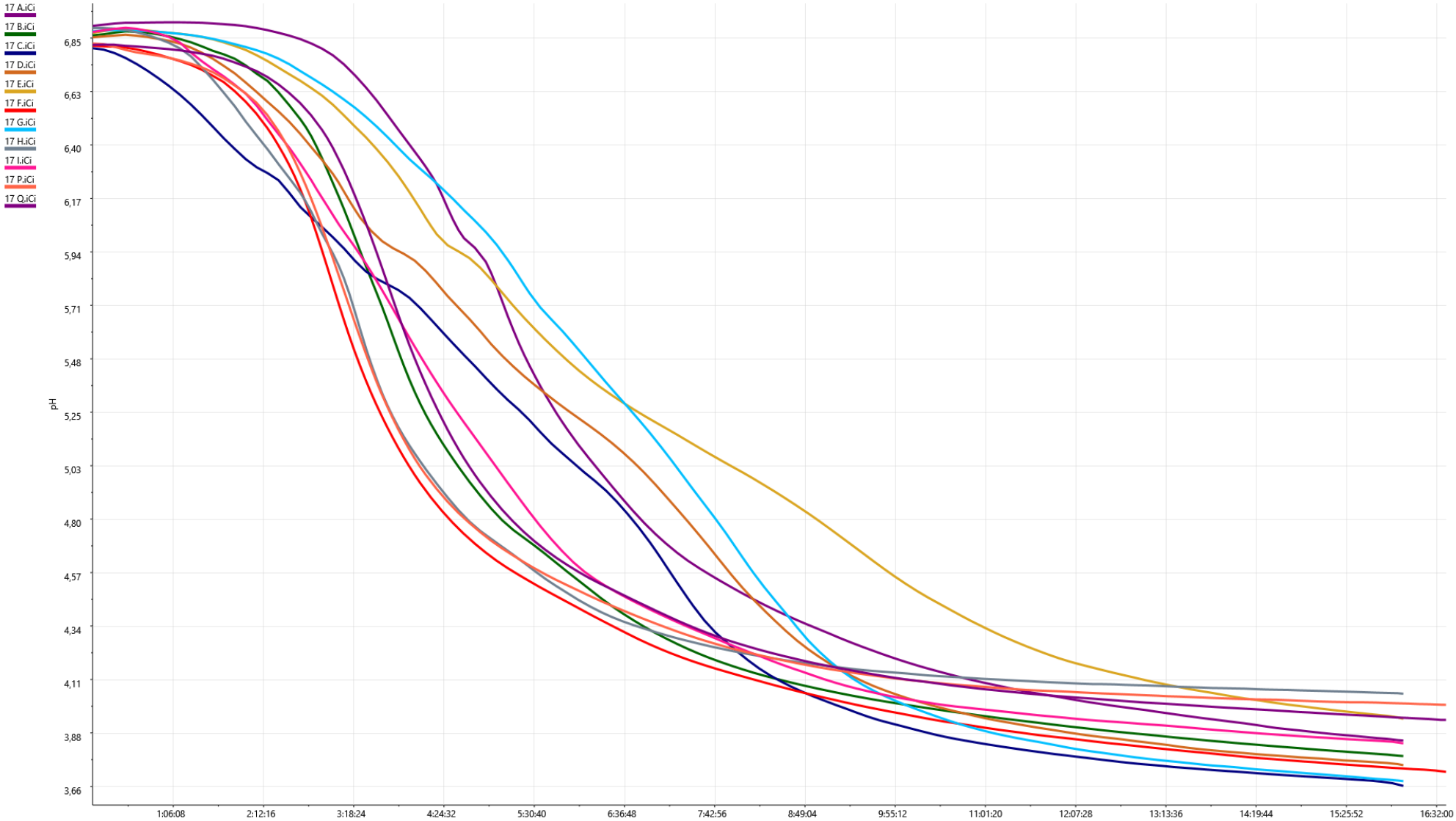

Figure 13. Fermentation curves of yoghurt fermented with starter cultures A (purple), B (green), C (dark blue), D (orange), E (yellow), F (red), G (light blue), H (grey), I (pink), P(deep orange) and Q (dark purple) in combination with protein 17.

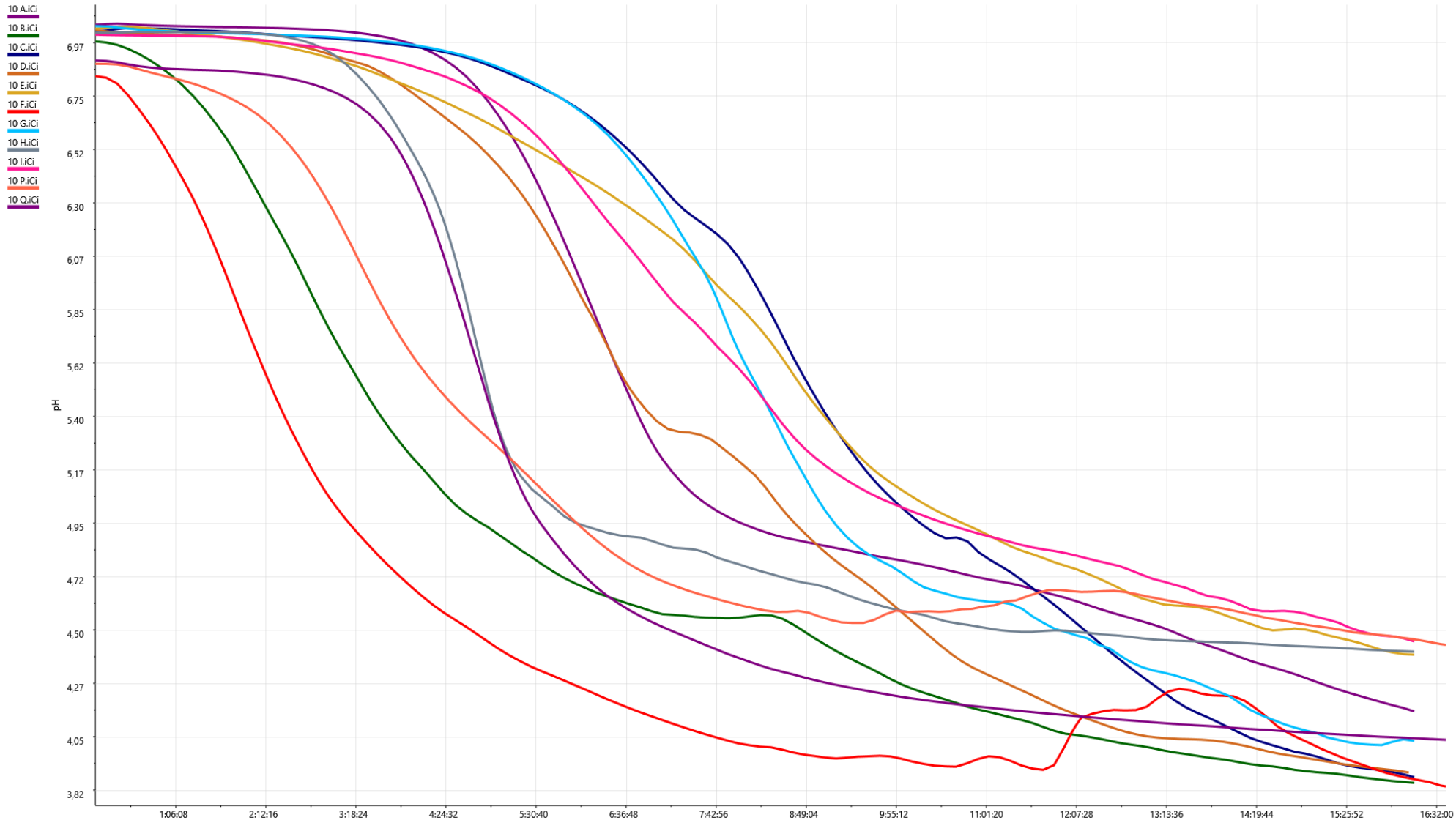

Figure 14. Fermentation curves of yoghurt fermented with starter cultures A (purple), B (green), C (dark blue), D (orange), E (yellow), F (red), G (light blue), H (grey), I (pink), P (deep orange) and Q (dark purple) in combination with protein 10.

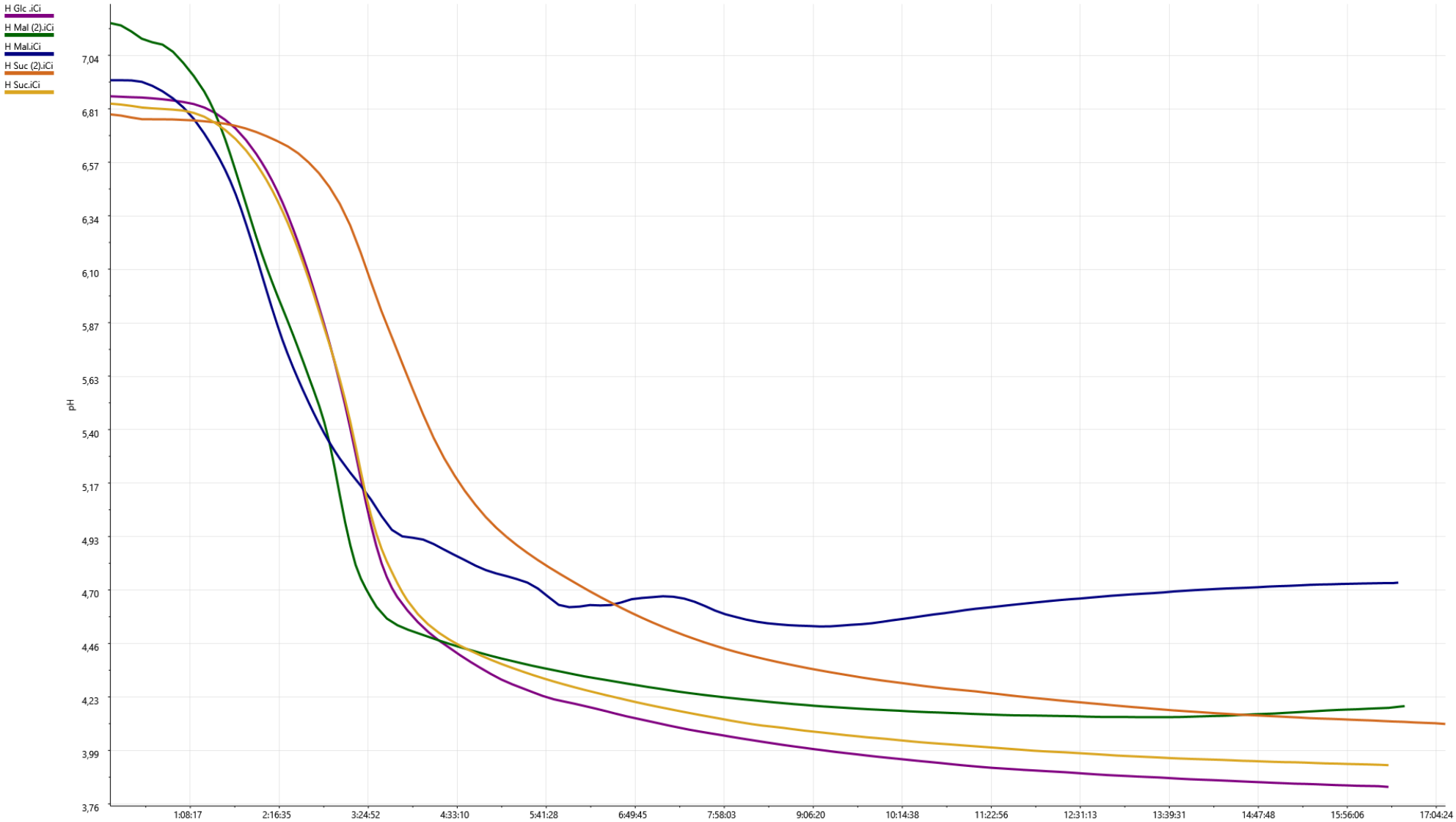

Figure 15. Fermentation curves of oat base fermented with starter culture H and three different energy sources. Glc = glucose, Mal = maltose and Suc = sucrose.

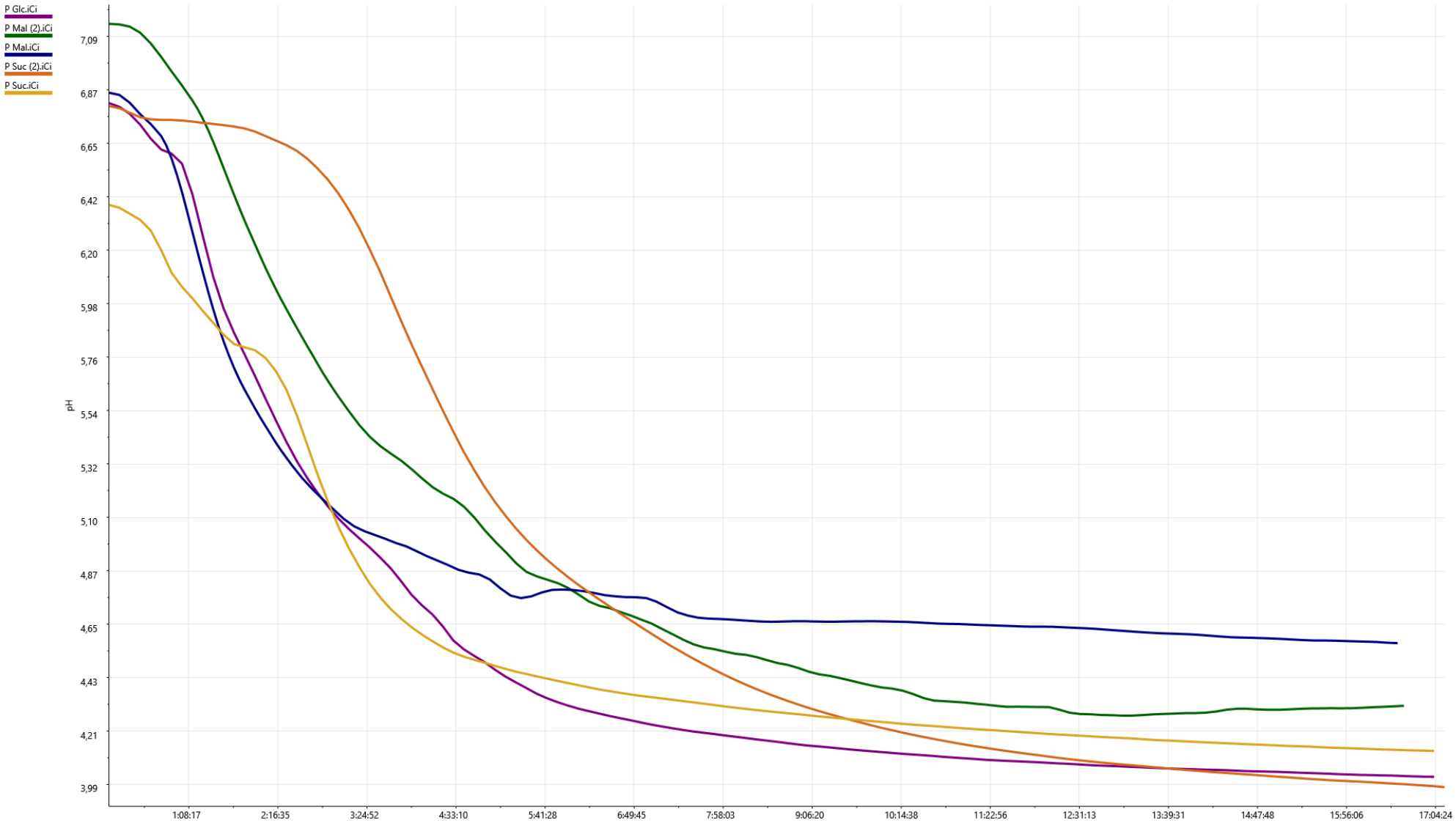

Figure 16. Fermentation curves of oat base fermented with starter culture P and three different energy sources. Glc = glucose, Mal = maltose and Suc = sucrose.