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Microcalorimetry as a Tool for Monitoring Food Fermentations

Martha Cuenca*[a] Benjamin Romen[b], Giacomo Gatti[b], Marco Mason[b], Matteo Scampicchio[b]

[a] Escuela de Ingeniería Química, Pontificia Universidad Católica de Valparaíso, Av. Brasil 2162, Valparaíso, Chile
[b] Faculty of Science and Technology, Free University of Bozen, Piazza Università 1, 39100, Bolzano, Italy
martha.cuenca@pucv.cl

Food fermentations are important to obtain different products such as bread, beer, mead, wine, yogurt, among other. The rate of fermentation is generally monitored by the measurement of simple variables like pH, Brix, titratable acidity and volume increase (in case of bread's dough). Isothermal microcalorimetry has been used to evaluate bacterial growth in medical, clinical, environmental and food fields. This work aims to show the potentiality of isothermal microcalorimetry as a tool for monitoring different fermentations. Yogurt fermentations were performed on 100 mL milk in isothermal conditions at 45°C with types of pasteurized milk (cow and goat), fermented by two activated commercial yogurt starters (with a different combination of *Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus* and a dose of 0.4 % w/v). Apricot juice fermentations were performed on 4 mL glass vials (15 Bx, pH 4.5) in isothermal conditions at 15°C with six different type of commercial yeasts *(Saccharomyces cerevisiae* with a dose of 0.3 % w/v*)*. Dough fermentations were performed on 4mL Hastelloy vials in isothermal conditions at 30°C with different flours (wheat, cornstarch, teff, commercial gluten free mixture and buckwheat) and commercial bread yeast *(Saccharomyces cerevisiae* with a dose of 1.0 % w/w*)*. All fermentations evaluated show a different behaviour for heat flow and accumulated heat: (1) for yogurt has a strong influence related to kind of milk as well as starter used. (2) for apricot juice has a strong influence related to kind of yeast used as well as its concentration. (3) for dough has a strong influence related to kind of flour used as well as the inclusion of wheat flour. These results confirm isothermal calorimetry can be combined with other techniques in order to be useful for monitoring different industrial fermentations and evaluating changes in formulation and process

1. Introduction

Yogurt is a product obtained from sanitized milk fermented at temperatures above 40ºC by the action of *Lactobacillus delbrueckii ss. bulgaricus* and *Streptococcus thermophilus salivarius ss*, its sensory characteristics not only depends on the type of milk used, but also on the starter used as well as the temperature of the process (Tamine & Robinson 1999). Manufacturing techniques have been modernized during the years in terms of the equipment used and the purity of the starter cultures; however, the fermentation process remains the same.(Tamine & Robinson 1999) .
Alcoholic fermentation can be defined as a process of anaerobic metabolism of yeast; it is the basis of spirits production. During alcoholic fermentation, yeasts convert fermentable sugars (glucose, fructose and sucrose) into ethanol and carbon dioxide. Even complex carbohydrates (maltose, starch, etc.) can be metabolized by following enzymatic reactions. The ability of yeast to produce alcohol and carbon dioxide depends on environmental factors such as the raw material, biological and technological factors etc. Temperature has great influence on the speed of propagation and fermentation capacity and the products of their secondary metabolites such as volatile organic compounds. Then, the amount of fermentable sugars such as glucose and fructose directly effects on the amount of alcohol obtainable. Also the species of yeast used in fermentation has its importance. The most widely used yeast is *Saccharomyces cerevisiae*.
Bread is a basic foodstuff obtained generally from a fermented dough and then baked. Generally, this dough is prepared using a mixture of wheat flour, water and yeast (*Saccharomyces cerevisiae*). Wheat (*Triticum spp.*) is a cereal which has gluten, a general name for a group of proteins that helps to maintain shape and texture

in foods. (Fasano & Catassi 2001). Celiac disease is a syndrome characterized by damage of intestinal mucosa in genetically susceptible subjects which cannot eat gluten. A gluten-free diet includes rice, wild rice, corn (maize), sorghum, millet, buckwheat (kasha), beans, peas, and bean flours quinoa, potato, soybean, tapioca, amaranth, teff, nuts, fruits, milk (cheeses), plain meat, fish, egg, (Fasano & Catassi 2001). There are a lot of bread-like products by using different raw materials and trying to keep similar sensorial characteristics. During dough fermentation starch is converted into simpler sugars, which then are consumed by yeast. In spite of other kinds of flours are used to prepare doughs, fermentation takes place but it can be seen different changes during the dough fermentation because of the absence of gluten.

Calorimetry has been used during the past 50 years for the experimental study of bacterial growth (Walker & Forrest 1964). Each process, chemical, physical and biological, causes both, production and consumption of heat. Heat flow calorimetry is a technique that measures directly the heat flow released or absorbed by a chemical reaction and it provides an accurate reaction fingerprint using a constant-area thermopile comprised of hundreds of temperature sensors. It can provide useful real time information about the yield, growth rate or the stoichiometry of a specific biological process and it is used to monitor bacterial activity (Braissant et al. 2013; Braissant et al. 2015), grapes withering (Morozova et al. 2016), and antioxidant capacity (Kamrul et al. 2015). The main advantage of this technique is that is a direct and continues measurement of the process providing real-time data during the whole process. It does not require any pre-treatment of the sample and is not destructive or invasive.

In spite of yogurt, fermented musts and dough are traditional products, there are few works related to monitoring their processes without sample treatment. So, calorimetry can be a powerful analytical tool for fermentations study.

2. Materials and methods

2.1 Yogurt fermentations

Four different yogurt fermentations (1-4) were prepared by using two types of pasteurized whole milk (cow and goat) and two commercial starters FD-DVS-YC-380-YoFlex (Starter 1) and FD-DVS-YF-L812-YoFlex (Starter 2) (different combination of *Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus* from CHR Hansen, Denmark). First fermentation (1) was performed with cowmilk and starter 1. An inoculum of Starter 1 was added as fermenting agent with a concentration of 0.04% (w/v). The other three fermentations were performed similarly, but by using cowmilk and starter 2 (2); goatmilk and starter 1 (3) and, goatmilk and starter 2 (4).

A single batch of pasteurized whole milk (cow or goat) was carried out by using a chemical reactor (Chemical Process Analyzer, Syrris, Royston, United Kingdom), with a total volume of 100 mL. The lid of the reactor was adapted to host a pH electrode to monitor it continuously, along the process. The reactor was closed gas-tight and drawn in a water bath. A peltier system allowed the fine control of isothermal conditions during fermentation. Fermentations trials were performed at 45 ± 0.1°C for 7h in triplicate. Measurements of heat flow (W) and pH were taken each 10 seconds.

2.2 Apricot juice fermentations

A commercial standardized apricot juice (pH of 4.5 and 15 Bx) was fermented. Six different commercial strains of lyophilized *Saccharomyces cerevisiae* were used as fermenting agents with a dose of 0.3% w/v: (a) Filtraferm® Spiri Aroma, (b) Lalvin EC-1118; (c) Enoferm® SIMI WHITE; (d) Cross (X) Evolution YSEO; (e) Uvaferm BC; (f) Feromol Arôme Plus. All yeasts were rehydrated in juice at 37°C for 15 min. Fermentations were conducted in 4mL completed seal glass vial at 15°C constant temperature in a multichannel microcalorimeter TAM III model 421 (TA Instruments, New Castle, United States). All trials were performed in triplicate.

2.3 Dough fermentations

It was used commercial lyophilized bread yeast (*Saccharomyces cerevisiae*), salt (NaCl), sunflower oil, and flours. Five different flours (wheat, cornstarch, teff, commercial gluten free and buckwheat) were used to prepare doughs. Then, separated doughs were prepared including 50% (w/w) of wheat flour (teff, commercial gluten free and buckwheat). All doughs were prepared by using a basic recipe and a bread homemaker machine (Princess Household Appliances, Lainate Italy). Every dough was prepared separately and after obtaining it, frozen (-18°C) to start fermentation measurements simultaneously. Table 1 shows the dough's basic amounts (% (w/w)) used to prepare doughs. All fermentations were performed for 15 hours by triplicate. All fermentation trials were performed in the same microcalorimeter of 2.2, evaluated by triplicate, by putting 800±10 mg of dough at 30°C for 15 hours in a 4 mL Hastelloy vial.

Table 1. Basic dough recipe used to obtain different doughs

Ingredient	Percentage (w/w)
Flour	60.7
Sunflower Oil	2.8
Water	34.8
Yeast	1.0
Sodium chloride	0.7

2.4 Data analysis

All statistical analysis were done with OriginLab 2015 (OriginLab Corporation Northampton, USA), like one-way ANOVA ($p<0.001$), Tukey test ($\alpha = 0.05$) and Principal Component Analysis.
Heat was obtained by a numerical integration of heat flow (W) taking into account the fermentation time. Heat is released as a consequence of microbiological activity (Braissant et al. 2013), and total heat can be modeled by using a modified Gompertz model (Eq. 1).

$$Q\ (J) = A_s * exp[-exp\left(\mu_{max} * \frac{e}{A_s}(\lambda - t) + 1\right)] \quad (1)$$

where, Q is the total heat (J), A_s is the asymptotic value relative to the microbial population (J), μ_{max} is the maximal specific heat flow (W) and λ is the lag phase time (s).

3. Results and discussion

3.1 Yogurt fermentations

Figure 1 (a) shows behaviour for F1, where pH decreases while heat flow presents a Gaussian behaviour related to the action of the microorganisms during fermentation, which it is confirmed by accumulated heat trend (Kabanova et al. 2012; Braissant et al. 2013; Braissant et al. 2015). In case of isoelectric point for milk (pH 4.5), it was reached at different fermentation time depending on milk and starter used. Comparing, F3 was faster than F1, while the slowest fermentation was F2. These differences are probably due to the internal protein structure in two milks because of its different amino acids assembly sequence and, as well as, goat casein has been associated with a lower mobility in an electrophoretic field (Bruhn & Roden 2015). In case of heat flow, Figure 1 (b) shows F4 as the fastest fermentation, although F3 presents the maximal reached heat flow, probably due to differences on the starters and their behavior within different food matrixes. These facts were also supported by the total amount of heat (J), as it is reported in Table 2, where F1 and F3 show similar values higher compared to those from F2 and F4. Table 2 presents also Gompertz parameters for accumulated heat by using Eq.1. These parameters confirm that heat generation depends strongly on starter used because of similar values obtained for parameters for F1 and F3, as well as F2 and F4, but also on the type of milk as shown by lag phase duration parameter.

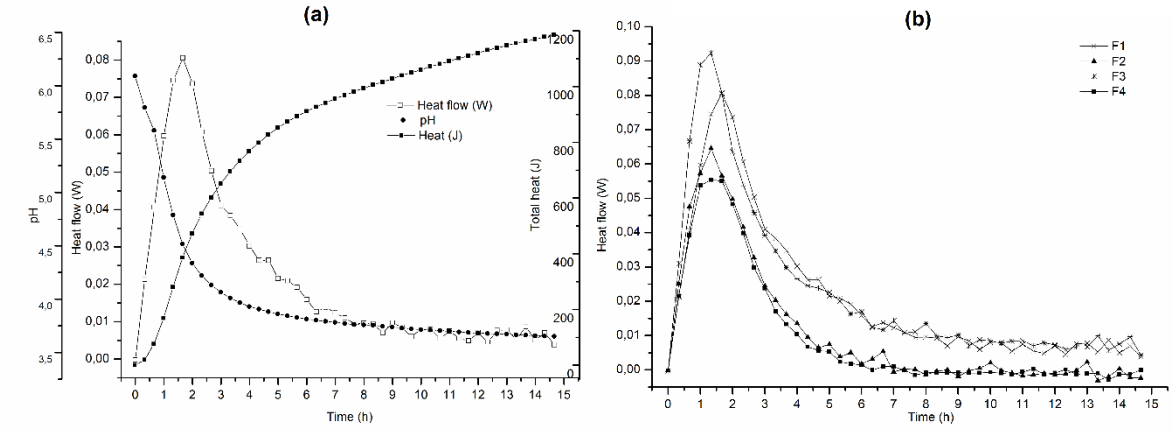

Figure 1. (a) Heat flow, pH and total heat profile for yogurt fermentation 1 (b) Thermogram of heat flow for all yogurt fermentations

Table 2. Parameters for each fermentation obtained by ANOVA ($p< 0.05$) and Gompertz parameters for accumulated heat for yogurt fermentations

Fermentation	Calorimetric parameters				Gompertz model parameters			
	Time to reach pH 4.5 (h)	Max heat flow (mW)	Time to reach maximum heat flow (h)	Total accumulated heat (J)	As (J)	Lag phase duration λ(h)	Max specific heat flow (W) x10^5	R^2
1	2.21±0.01 a	86.7±5.3 a	1.62±0.01 a	905±149 a	919±1 a	0.61±0.01 a	7290 ± 1 a	0.997
2	3.71±0.03 b	66.5±9.0 b	1.22±0.02 b	569±30 b	589±1 b	0.44±0.01 b	5990 ± 1 b	0.999
3	1.69±0.02 c	95.2±9.2 a	1.21±0.02 b	956±7 a	919±1 a	0.36±0.01 c	7520 ± 1 c	0.994
4	3.03±0.01 d	59.9±0.9 b	1.18±0.03 b	517±75 b	531±1 d	0.49±0.01 d	5680 ± 1 d	0.999

Different letters in each column indicate significant differences at 95% confidence level as obtained by the Tukey test

3.2 Apricot juice fermentations

Figure 2 shows all fermentation threshold for every yeast. (a) shown the biggest peak of thermal power subsequent has (b), (f), (c) and (d) while (e) presented the lowest thermal power (probably this yeast has a higher optimal fermentation temperature). All parameters are presented on Table 3. Growth rate during exponential phase presents a characteristic threshold. (a) finishes first the fermentation after 3.5 days. Other yeasts finish fermentation at the same order when starting the exponential phase ((a) < (f) < (b) < (c) < (d) < (e)). Related to the total heat produced during fermentation, the first is (a) and the rest are (c) < (f) < (b) (d) < (e). Similar to heat flow results, (e) presented the smallest value which is similar to results obtained by Ciani et. Al. 2010 (Ciani et al. 2010). All results observed are according to results reported by (Kabanova 2013), who shows the possibility of monitoring bacteria metabolism by microcalorimetry on solid and liquid matrixes.

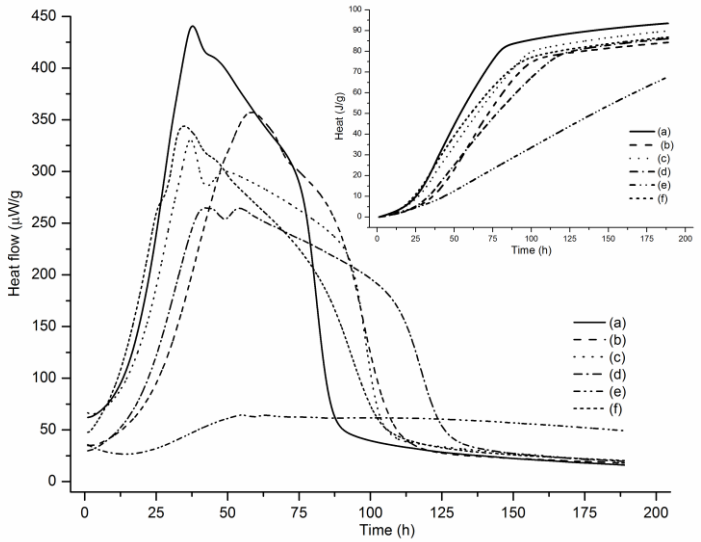

Figure 2. Thermogram of Heat flow (µW/g) and Heat (J/g) vs time at different yeasts in apricot juice

Table 3. Value of Lag time, Initial time, Final time, Enthalpy, Maximal rate, and Maximal Power for different commercial yeasts fermentations in apricot must.

Fermentation	Lag time (h)	Initial time (h)	Final time (h)	Enthalpy (J/g)	Max rate (µW/h)	Max power (µW)
(a)	1.5±0.1 a	12±0.1 a	84±0.1 a	6.0±0.2 a	19.6±0.1 a	364±1 a
(b)	1.5±0.1 a	18±0.1 b	108±0.1 b	4.6±0.1 b	10.5±0.2 b	352±1 b
(c)	1.5±0.1 a	12±0.1 a	113±0.1 c	5.2±0.1 c	13.2±0.2 c	355±1 c
(d)	1.5±0.1 a	14±0.1 c	124±0.1 d	4.5±0.2 b	10.1±0.1 b	270±1 d
(e)	1.5±0.1 a	nd	nd	2.4±0.1 d	1.28±0.2 d	45.1±1 e
(f)	1.5±0.1 a	2±0.1 d	105±0.1 e	5.3±0.2 c	13±0.1 c	330±1 f

Different letters in each column indicate significant differences at 95% confidence level as obtained by the Tukey test

3.3 Dough fermentation

Figure 3 (a) shows the variation of heat flow (W/g) including different flours and (b) with 50% of wheat flour. All doughs present a different behavior of heat flow because all the compositions of flours are different. In case of teff, it presents the maximum heat flow around 2 hours, while corn starch presented the smallest peak of the heat flow around 1.5 hours, probably because it only contains starch without another component and also because during refining starch process all amylases enzymes (normally present in pericarp corn seed) which are responsible of starch degradation to fermentable carbohydrates available for yeast are not present. Wheat dough present a maximum heat flow similar to the commercial gluten free bread mix flour. Taking into account the inclusion of 50% of wheat, it is observed a different behavior of heat flow, showing characteristics due to the synergic interactions between flours.

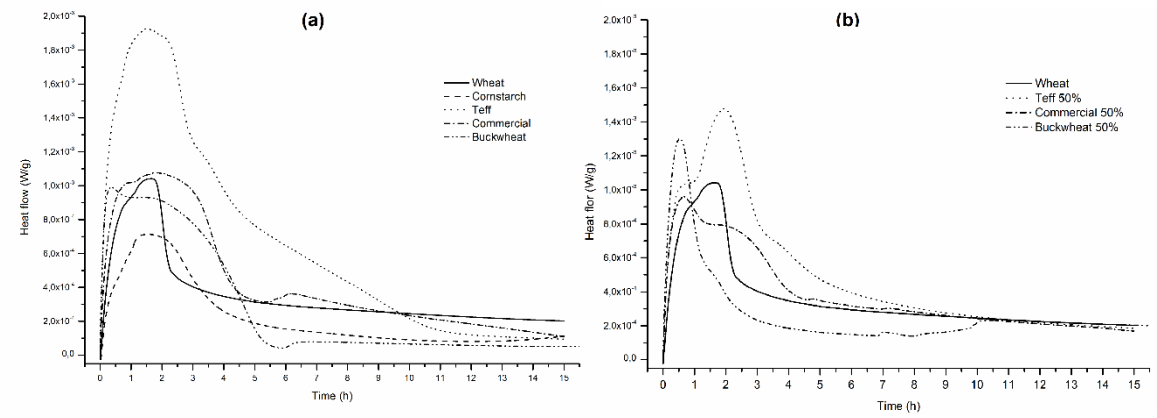

Figure 3. Behavior of heat flow of (a) different flours (b) 50% wheat flour addition dough fermentations.

Table 4 presents all microcalorimetry parameters obtained. In all cases there are significant differences expected because of the variation of type of flour. A PCA analysis was performed and a biplot showing PC1 (71.65%) and PC2 (96.51%) of score data was plotted (Figure 4). Doughs are completely different among them, and when 50% (w/w) of wheat flour is included their behavior is completely different. This analysis confirms microcalorimetry can classify dough when different kind of flour is used to prepare them.

Table 4. Microcalorimetry parameters for dough fermentations with different flours

Dough	Maximal heat flow (mW/g)	Time to reach maximal heat flow (h)	Entalpy (J/g)
Wheat	1.04±0.03 a	1.53±0.01 a	19.2±1.1 a
Cornstarch	0.71±0.01 b	1.45±0.01 b	12.0±0.3 b
Teff	1.93±0.04 c	1.57±0.01 c	35.7±1.4 c
Commercial	1.08±0.04 d	1.80±0.01 d	23.1±0.1 d
Buckwheat	0.99±0.01 e	0.35±0.01 e	15.6±1.4 e
Teff 50%	1.48±0.02 f	1.94±0.01 f	26.5±0.4 f
Commercial 50%	0.96±0.05 g	0.62±0.01 g	20.6±0.3 g
Buckwheat 50%	1.30±0.03 h	0.55±0.01 h	14.8±0.3 h

Different letters in each column indicate significant differences at 95% confidence level as obtained by the Tukey test

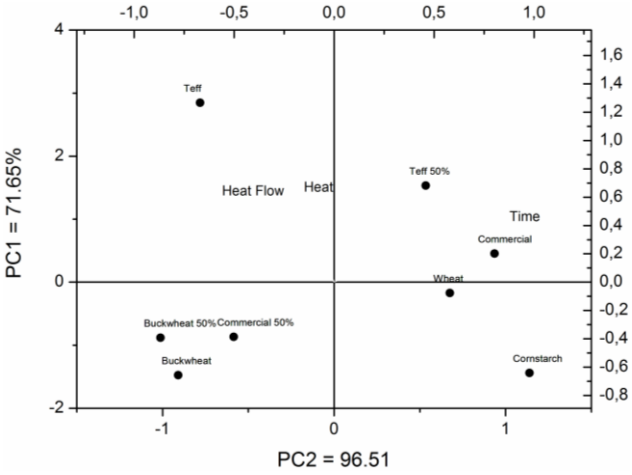

Figure 4. Biplot obtained by applying PCA for dough fermentations with different flours

4. Conclusions

Isothermal calorimetry is a technique which can be useful to evaluate lactic, ethanolic and dough fermentation according to changes like ingredients, type of microorganism and temperature. If it is combine with other analytical techniques will be useful to model kinetics fermentation and to take different decisions related to formulation and process conditions.

Acknowledgments

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