Technology Updates | Food

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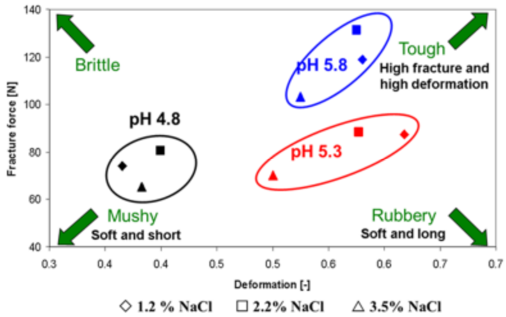
Design and development of Realistic food Models with well-characterized micro- and macro-structure and composition (DREAM)



Key external stakeholders:

Cheese and Dairy Industry

Practical implications for stakeholders:

The outcome/technology or information/recommendation is:

A procedure for the preparation of a semi-hard rennet-curd model cheese in which composition is precisely controlled and which can be used to validate the growth/survival of microorganisms under different conditions.

A database on the effects of varying salt concentration and pH in model cheese on the survival of probiotic bacterial strains and on the chemical and rheological properties.

A data base on the survival of probiotic bacterial strains in full-salt and reduced-salt Cheddar cheeses during maturation

Main results:

1. A model cheese-making system was designed for the manufacture of semi-hard rennet-curd cheese (~47% dry matter, 18% protein, protein-to-fat ratio ~1) in which salt (1.2, 2.2 or 3.5%) and pH (4.8, 5.3 or 5.8) could be systematically controlled. The model cheeses were prepared from concentrated dispersions of micellar casein in an aqueous-based solvent comprised of water, fat and lactose.
2. The values for the coefficient of variation for salt, moisture, salt-in-moisture and pH in the model cheeses were ≤ 5%, indicating a high degree of reproducibility.
3. The survival of probiotic bacteria *Bifidobacteria* BB12 (BB12), incorporated into the model cheese at a level of ~ 10^8 cfu/g, was independent of variations in NaCl and pH at ripening times up to 96 d. However, after 150 d storage, the mean count of BB12 had decreased significantly (to ~ $10^{5.6}$ cfu/g) in the high-salt (3.5%) high-pH (5.8) cheese but not in the other cheeses.
4. The survival of probiotoc strain *Lactobacillus casei* LC-01 (LC-01), incorporated into the model cheese at ~ 10^8 cfu/g, was independent of pH variation in the high salt cheese (3.5%) at ripening times up to 47 day but decreased significantly to < 10^6 cfu/g in the low pH cheese (pH 4.8) after longer ripening times (96-150 day). However, the survival of LC-O1 was not affected by pH in the cheese with lower salt levels (1.2 and 2.2%).
5. A study on the survival of probiotic strains (BB12 and LC-01) in Cheddar cheese showed that both strains grew in the cheese during ripening, from ~ 10^8 cfu/g at 1 day ~ 10^{10} cfu/g at 60 day, and were unaffected by salt content of the cheese (1.4 %, 1.8 %).

Opportunity / Benefit:

The research makes available to the dairy industry a database on the effects of salt and pH on:

- the survival of two probiotic bacterial strains in a semi-hard model cheese (47% dry matter) and Cheddar cheese
- the chemical, rheological and viscoelastic properties of model cheese.

Collaborating Institutions:

INRA; ADRI Development; Campden BRI; Consiglio Nazionale delle Ricerche, Instituto di Scienze delle Produzioni; Actalia- Produits Laitiers; KOKI, Központi Élelmiszer-tudományi Kutatóintézet; INRA Transfert; Campden, BRI;CNRS, Centre National de la Recherché Scientifique; IRTA, Institut de Recerca I Tecnologia Agroalimentàries Alimentari; IFR, Institute of Food Research; TI Food and Nutrition, Stitching Top Institute Food and Nutrition; United Biscuits (UK) Limited; VTT, Valtion Teknillinen Tutkimuskeskus; Soredab, Soredab SAS; University of Ljubljana; Wageningen University.

Contact Tim Guinee Email: tim.guinee@teagasc.ie

Teagasc project team: Dr. Tim Guinee (PI)
Dr. Brian Byrne
Dr. Ivo Piska
Ms. Catherine Mullins

External collaborators: None

1. Project background:

Cheese is a very diverse product with greater than 500 different varieties. Consequently, cheese differs tremendously in composition, structure, rheology and suitability as a matrix to host different bacteria, depending on variety. Hence, there is a requirement for generic realistic cheese models that can simulate this complexity and, thereby, facilitate evaluation of the impact of changing composition or processing conditions on the characteristics of cheese. Cheeses made using conventional technology can be quite variable in composition, texture and sensory properties. Such variation is in turn associated with variations in milk composition (e.g., lactose and casein contents), starter culture activity, manufacturing conditions (e.g., rennet-to-casein ratio), and partition of milk components (e.g. calcium, sodium chloride) between the curd and whey (which accounts for ~ 90% of the milk volume). Such inadvertent variation in cheese composition can make it difficult to establish the precise effects of parameters such as salt and pH on cheese properties.

The project aimed to develop a model cheese in which the effects of salt and pH, which vary widely across the large array of different cheese varieties, could be studied without the confounding effects of variations in other parameters such as weight ratio of cheese serum-to-cheese matrix, moisture content and calcium level. The principal focus was on the effect of salt and pH on the survival of probiotic bacterial strains and on the physico-chemical and rheological properties of the cheese.

2. Questions addressed by the project:

- Do variations in salt content and pH as found across the spectrum of different cheese varieties affect the survival of commonly used probiotic strains in cheese?
- How do variations in salt and pH affect the biochemical and physical properties of cheese in situations where the ratio of solvent (water and dissolved solutes)-to-solid cheese matrix (protein, fat and colloidal ash) is maintained constant as salt and pH are changed?

3. The experimental studies:

Development of a model cheese system for variation of salt and pH

A model cheese system was developed in which the solvent quality (salt content and pH) was changed while retaining a fixed weight ratio of solvent (water, soluble calcium and phosphate, lactose, lactic acid and salt) to matrix (fat, casein and colloidal minerals) were kept constant. The model cheese (~47% dry matter, protein-to-fat ratio ~1) was developed by dispersing micellar casein in an aqueous-based solvent comprised of water, fat and lactose. The resultant dispersion (pre-cheese), which was a liquid pre-cheese, was pasteurised at 80 °C, and cooled to ~ 35 °C, inoculated with coagulant (chymosin), acidifying agent (glucono-δ-lactone) and salt. The treated pre-cheese was filled into the final package/mould and incubated at 32 °C to allow in-situ gelation and transformation into the final cheese. The reproducibility of the model system in the manufacture of cheeses with different pH salt and pH values was evaluated by undertaking three separate trials on different occasions.

Evaluation of the effect of salt and pH on the survival of probiotic bacteria in model cheeses

Model cheeses with varying levels of salt (1.2, 2.2 or 3.5%) and pH (4.8, 5.3 or 5.8) were made in triplicate by varying the level of salt and glucono-δ-lactone added to the model cheese system. Probiotic bacteria strains *Bifidobacteria BB12* (BB12) and *Lactobacillus casei* LC-01 (LC-01) (Chr. Hansen Ireland Limited, Little Island, Cork) were inoculated into the model pre-cheeses at a level of ~ 10^8 cfu/g prior to coagulation. The population of the probiotic strains cheeses was monitored over a 150-day storage period at 4 °C. BB12 were enumerated on *Bifidobacteria* Selective Media with added BSM antimicrobial supplement (BSM: Fluka Analytical, Sigma Aldrich) incubated an-aerobically (Anaerocult® A; Merck KGaA, Germany) at 37 °C for 3 days. LC-01 was enumerated on LBS agar (Becton Dickinson Co, Cockeysville, USA) incubated at 37 °C for 4 days.

Contact Tim Guinee Email: tim.guinee@teagasc.ie

Survival of probiotic bacteria in full-salt and reduced-salt Cheddar cheeses
Full salt (1.8%) and reduced salt (1.4%) Cheddar cheeses were made in triplicate using the following treatments (Control, PB BB12, PB LC-01):

(a) Control full-salt (FSC) and reduced-salt (RSC) Cheddar cheeses, made with a commercial mesophilic lactic acid starter culture (R-704);

(b) PB BB12 full-salt (FSC) and reduced-salt (RSC) Cheddar cheeses, made with R-704 plus probiotic bacteria *Bifidobacteria* BB12 (BB12), inoculated into the cheesemilk at a level of $10^{7.8}$ cfu/ml;

(c) PB LC-01 full-salt (FSC) and reduced-salt (RSC) Cheddar cheeses, made with R-704 plus probiotic bacteria *Lactobacillus casei* LC-01 (LC-01), inoculated into the cheesemilk at a level of $10^{7.6}$ cfu/g.

The population of starter culture and probiotic bacteria in the cheese were enumerated over a 180-day ripening period. The cheeses were also evaluated for composition at 14 d, rheology characteristics, and grading scores at 6 month.

Effect of salt and pH on the chemical and rheological properties of model cheese
Model cheeses with varying salt content (1.2, 2.2 or 3.5%, w/w) and pH (4.8, 5.3 or 5.8) were manufactured in triplicate, as described above. The cheeses were stored for 5 days at 4 °C and analysed for:

- Gross composition (fat, protein, moisture, salt, pH, Ca);
- Protein hydration, as determined from the level of expressible serum obtained on centrifugation of grated cheese at 12500 x g at 20°C
- Texture profile analysis, using a two-bite compression test to 30% of samples original height at a rate of 1 mm/s, which enabled the calculation of fracture force (force required to fracture, bite 1), hardness (force at maximum deformation, bite 1), and chewiness (product of hardness x cohesiveness x springiness, using analysis of bites 1 and 2).
- Viscoelastic changes on heating from 25 to 85°C at a rate of 3K/min, using low amplitude strain oscillation, to obtain elastic modulus (G'), phase angle (δ) -an index of fluidity of heated cheese.

4. Main results:

Survival of probiotic bacteria in model cheeses with different salt and fat levels

- The model cheese had the following composition: solvent (moisture, GDL and NaCl), 60%; protein, 18%; fat, 16.5%. The model cheese-making system showed a high degree of reproducibility (coefficient of variation < 5%) for levels of salt, pH and moisture for cheeses with a wide range of salt (1.2 – 3.5%, w/w) and pH (4.8 – 5.8) as found in different cheese varieties.
- *Bifidobacteria* BB12-inoculated cheeses maintained their target pH values at all salt levels and BB12 survival was independent of variations in salt content and pH, with counts between 10^7 and 10^8 cfu/g in all cheeses at 96 d. However, the count ($10^{5.6}$ cfu/g) of BB12 in the high-salt (3.5%) high-pH (5.8) cheese after 150 d storage at 4 °C was lower than that (≥ 10^6 cfu/g) associated with probiotic foods. However, this reduction was not observed in the high-salt cheeses with lower pH values (4.8 and 5.3) which had mean counts of $10^{6.5}$ – $10^{8.5}$ cfu/g after 150 d storage.
- The pH of the LC-01 inoculated model cheeses with target pH values of 5.8 and 5.3 and containing 1.2% salt decreased to 4.7 – 4.8 after 1 day, while the pH of the corresponding cheeses with 2.2% NaCl had decreased to 4.7 – 4.8 after 96 day. The decrease in pH of the latter cheeses suggests that LC-01 grew during storage at 4 °C and metabolized lactose to lactic acid. In contrast, there was no change in the pH of LC-01 cheeses with 3.5% NaCl. The levels of *L. casei* LC-01 recovered from all LC-01 inoculated cheeses (that maintained their target pH) remained at levels of ≥10^6 cfu/g up to 150 d storage.

Survival of probiotic bacteria in full- and reduced-salt Cheddar cheeses

- The addition of probiotic bacteria significantly increased the levels of moisture, moisture-in-non-fat substances, and fat-in-dry matter, and reduced levels of salt and salt-in-moisture of both the full-salt cheese (FSC) and reduced-salt cheese (RSC). The pH of the PB LC-01 FSC was significantly lower than that of the control FSC or the PB BB12 FSC, while pH of the PB LC-01 RSC and PB BB12 RSC were significantly lower than the corresponding control RSC. The latter trend may reflect the high levels of moisture and MNFS, and lower S/M content of the probiotic cheeses, especially the PB BB12 RSC. Reducing the salt content significantly increased the contents of moisture and fat, while significantly reducing salt-in-moisture content and pH. The lower pH of the reduced-salt cheeses reflects their higher moisture content which would lead to a higher level of lactic acid.
- The counts of probiotic-bacteria in the PB BB12 FS, PB BB12 RS, PB LC-01 FS and PB LC-01 RS cheeses increased from ~ 10^8 cfu/g in the cheese milk after inoculation to 10^9 - 10^{10} cfu/g in

Contact Tim Guinee Email: tim.guinee@teagasc.ie

the cheese at day 1. Over the 180 day ripening period, the probiotic strains decreased by ~ 1 to 2 log units, reaching mean levels of ~ $10^{7.5}$ at day 180. As counts were > 10^6 cfu/g, the 180-day old ripened cheese can be referred to as probiotic cheese. Salt content did not significantly influence initial counts (on day 1) or the survival BB12 or LC-01 in Cheddar cheese. These results concur with those from the model system which found that salt levels in the range 1.2 – 2.2% (inclusive of the range in FS and RS cheddar cheeses: 1.4 – 1.8%) at pH values in the range 4.8 – 5.3 (inclusive of the range in FS and RS cheddar cheeses: 5.06– 5.21) had little effect on the survival of BB12 or LC-01.

- Despite the differences in composition, the probiotic culture did not significantly affect cheese hardness, fracture stress or fracture strain over a 6 month ripening period, apart from at 60 day when the fracture strain of probiotic cheeses was lower than that of the control cheeses.
- Relative to control FSC cheese, the BB12 FSC was considered very acceptable, and had a sweetish flavour note, balanced flavour, and creamy–smooth texture. Conversely, the PB LC-01 FSC cheese had a strong savoury, slightly rancid flavour more typical of very mature aged Cheddar (compared to the control cheese). Reducing the salt content resulted in all cheeses being described as being sharper, more acid, slightly bitter, and less balanced.

Effect of salt and pH on the chemical and rheological properties of model cheese

- For all salt levels, increasing pH from 4.8 to 5.3 significantly reduced cheese hardness, while increasing pH from 5.3 to 5.8 had little effect. Altering the salt concentration in final cheese did not significantly affect the hardness.
- Chewiness was highest at pH 5.3, and decreased with increasing salt concentration at all pH values.
- A plot of fracture force versus deformation at fracture point yielded a texture map indicating how the pH and salt level separated cheeses (Fig. 1). Model cheeses with low pH had low fracture force and deformation (fracture strain), indicating a tendency to be relatively soft and crumbly (short consistency). Conversely, the high pH cheeses had higher fracture force and deformation, suggesting an increasing tendency to tough, hard, rubbery texture as pH was increased. Increasing the pH from 4.8 to 5.3, which is typical of that of many hard cheese varieties, increased the deformation required at fracture, signifying an increase in rubberyness but not in hardness. Compared to pH, the effect of increasing salt content was relatively small, though statistically significant.
- Salt and also pH significantly altered the value of G' at 25°C. Lowest values were found at pH 5.3 at all salt concentrations and highest values at pH 4.8.
- Increasing pH increased the maximum phase angle (index of fluidity of the melted cheese) on heating the cheese from 25 to 82 °C. Increasing the salt concentration led to an increase in the temperature at which phase angle attained a maximum value. In practical terms, the results indicated that increased pH increased the fluidity of the melted cheese, while increasing salt from 1.2 to 3.5 % increase the temperature required to obtain the maximum fluidity of the melted cheese.
- Protein hydration decreased significantly as pH was increased in the region 5.3 to 5.8, and as salt was reduced from 3.5 to 1.2%.

5. Opportunity/Benefit:

The research provides an extensive database on the effects of varying salt level in the range 1.2 to 3.5% and pH in the range 4.8 to 5.8 on the survival of common probiotic bacterial strains in cheese.

6. Dissemination:

Dream, Project Book of Results: Design and Development of Realistic Food Models with well-characterized micro-and macro-structure and composition (2013). University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, Slovenia.

Byrne, B., Jordan, K. and Guinee, T. P. (2012). Effect pH and NaCl on the survival of probiotic cultures in a model cheese system. In proceedings of 8th Cheese Symposium.

Piska, I., Byrne, B. and Guinee, T. P. (2012). Effect of sodium chloride on the properties of a model cheese system. In proceedings of 8th Cheese Symposium.

7. Compiled by: T.P. Guinee

Contact Tim Guinee Email: tim.guinee@teagasc.ie