Manuscript Details

Manuscript number	TIFS_2017_556_R1
Title	STATUS AND DEVELOPMENTS IN ANALOGUE CHEESE FORMULATIONS AND FUNCTIONALITIES
Article type	Review Article

Abstract

Background: Analogue cheeses (AC) are homogeneous cheese-like matrices obtained by mixing water, oils/fats, proteins, emulsifying salts and other ingredients under heat and mechanical shear. These versatile products are used both directly for consumption and mainly indirectly as ingredients in several foods. Scope and approach: Increasing consumers' expectations, consumption habits, current lifestyles and cheese industry dynamism are factors driving the research towards the development of new cheese-like products and functionalities. This review describes the state of the art on AC formulations in relation to properties of the final product. Key findings and conclusions: Extensive data from research on AC highlight the current growing interest for the development of innovative functionalities to satisfy specific end-use applications. The outcomes of most investigations drew attention to the basic role of type and amount of ingredients to obtain a wide array of customized attributes. An insight on the role and the interactions among constituents of the formulation and the effect on textural, rheological, melting and sensory properties of AC was provided.

Keywords	Analogue cheese; Dry ingredients; Casein; Emulsifying salts; Functional properties.
Manuscript region of origin	Europe

Corresponding Author	MASOTTI FABIO
Corresponding Author's Institution	Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Università degli Studi di Milano
Order of Authors	MASOTTI FABIO, stefano cattaneo, Milda Stuknytė, Ivano De Noni
Suggested reviewers	Bartosz Solowiej, Paul L.H. McSweeney, dolores oriordan, Tim Guinee

Submission Files Included in this PDF

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January 29th, 2018

Dear Editor Prof. Fidel Toldra,

We thank You for taking care of the revision of our manuscript "Status and developments in analogue cheese formulations and functionalities" (Ref. No.: TIFS_2017_556) by Stefano Cattaneo, Milda Stuknytė, Ivano De Noni and myself to be considered for publication in *Trends in Food Science & Technology*.

We are grateful to Reviewers for amendments and suggestions to improve the scientific accuracy and the writing style. To reply comments we exceeded the editorial limit of 10,000 words (13,676 in the revised manuscript). Please, find attached an itemized list of responses in separately submitted files.

Thank you for your consideration.

Sincerely,

Fabio Masotti

Corresponding author

Università degli Studi di Milano,
Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente (DeFENS),
Via G. Celoria 2,
20133 Milan, Italy
E-mail: fabio.masotti@unimi.it
Phone: +39 02 50316665
Fax: +39 02 50316672

Response to Reviewer #1
Ref: TIFS_2017_556
Title: STATUS AND DEVELOPMENTS IN ANALOGUE CHEESE FORMULATIONS
Journal: Trends in Food Science & Technology

We are grateful to the Reviewer for several amendments to improve the scientific quality of the manuscript. To simplify reading, the original points raised by the Reviewer are in black bold and our answers are in black typeface after the initials AU:.
Changes in the revised manuscript are highlighted in grey.

1. **Suggested title: STATUS AND DEVELOPMENTS IN ANALOGUE CHEESE FORMULATIONS AND CHEESE FUNCTIONALITIES**
   The title given by the author – does not indicate dealing of functional properties of cheese for its end use application. Hence, the suggested Title.
   AU: We thank the Reviewer for Her/His suggestion and modified the title into "STATUS AND DEVELOPMENTS IN ANALOGUE CHEESE FORMULATIONS AND FUNCTIONALITIES".

2. **Abstract – Take into account sensory quality of analogue cheese too in Abstract**
   AU: We added the adjective "sensory" in the abstract (revised line 24).

3. **For cheese analogue flavouring, dairy cream or butter oil has been referred to – These ingredients can only contribute dairy flavour, but never cheese like flavour that is intended in cheese analogues.**
   AU: We modified the text as follows: "Generally, an important difference of AC in comparison to the natural counterpart is considered the bland flavor. The use of little amounts of cheese (<50 mg/kg of the final product, Fox et al., 2017a) is a way to overcome this defect and impart flavor. An alternative is the use of dairy cream instead of vegetable oil/fat." Revised lines 541–545.

4. **Authors have claimed 'Non-dairy analogue cheese has no commercial value' (Is this quoted by any author – such demeaning statement should be refrained from getting quoted)**
   AU: We thank the Reviewer for Her/His remark. We removed this statement quoted by Guinee, Caric and Kalab (page 381, Pasteurized processed cheese and substitute/imitation cheese products. In: Fox PF, editor. *Cheese: chemistry, physics and microbiology. Volume 2: major cheese groups*. 3rd ed. London , U.K.: Elsevier Applied Science. 2004) and modified the sentence as follows: "Non-dairy AC (largely obtained from vegetable proteins and fats) represent a market opportunity for vegan cheeses." Revised lines 50–51.

5. **Line 89 - What is meant by 'Clean of sliceability'?? (Line 89)**
   AU: We thank the Reviewer. Clean of sliceability is the functionality to cut cleanly AC into thin slices without fracturing or crumbling or sticking to cutting implement.

6. **Shredability and sliceability are two different parameters. Authors have dealt them as similar aspect.**
   AU: We thank the Reviewer for highlighting the error. Really, we intended to list some functional properties of unheated AC. We modified the sentence. "…sliceability and shreddability …". Revised line 101.

7. **Specify few important functionalities of some cheese varieties i.e. shredability, stretch,melt for Mozzarella cheese. Then discuss those functionalities that were obtained in Mozzarella cheese analogue (MCA). Without referring to specific functionalities associated with specific cheese types, the functionality of AC has been discussed.**
   AU: We thank the Reviewer for Her/His revision. We introduced a paragraph entitled "Functional properties of analogue cheeses *vs* natural cheeses and changes upon storage" dealing with this topic (revised lines 657–711).

8. **Line 98 – Ingredients used in AC formulation has been wrongly written as constituents.**
   AU: We thank the Reviewer for Her/His amendment and corrected the text accordingly.

9. **Line 100 – Mention about Ref. relating to use of Emulsifiers (other than ES) in preparing ACs.**
   AU: We introduced 2 Refs. concerning the use of emulsifiers (Jana & Upadhyay, 2001; Jana, Upadhyay, & Solanky, 2005). Revised lines 111–112.

10. **In some cases author is writing – a specific aspect has been reviewed extensively – then quoting only one Reference for such aspect!!**
    AU: We agree with the Reviewer and corrected the text.

11. **Under heading of Protein as constituent of AC, they mentioned usual range of protein to be 18-24%. In Table 2 they discussed protein up to 36% - why such high excessive protein was kept in cheese analogue – needs to be discussed properly.**
    AU: We thank the Reviewer in noticing the inconsistency of data reported. We introduced details in a new paragraph "Arimi, Duggan, O'Sullivan, Lyng, and O'Riordan (2012) reported a content of protein in AC up to 35%. The authors developed a microwaveable snack food characterized by low fat content in comparison to fried snacks. This imitation cheese-type product, is essentially a protein-based matrix containing resistant starch, expanding when microwaved to form a crispy product. The authors observed that increasing protein content in AC from 14% to 35% was paralleled by an increase in the volumetric expansion values from

390% to 440% after microwave heating. This phenomenon was explained by the fact that it is the hydrated protein matrix alone that stretches and swells under the pressure of steam during microwaving.”. Revised lines 150–157.

12. **Why discussing increased water content in cheese – for a specific variety limit is given for max. moisture content that can be kept! You just give the role played by water in AC. Do not narrate all research aspects in unrelated cheeses or basic aspects of cheese.**
    AU: We deleted some sentences related to basic aspects of cheese. Please, see Water Section

13. **Line 155 – AC heating (what does it imply – was it baking – write proper technological words)**
    AU: We thank the Reviewer for Her/His correction. We modified the text and moved the quotation of Hennelly et al. (2006) under Inulin Sections. Revised line 457.

14. **In few cases 60% moisture has been specified in AC – which type of cheese is that (needs to be specified). Recommend whether AC can be very well prepared for soft cheese varieties or for hard cheese types!**
    AU: We moved the reference of Noronha et al. (2007) in starch Section.
    “Noronha et al. (2007) in an AC with 52% moisture and 12% fat contents replaced about 50% fat, consisting of hydrogenated palm oil and rapeseed oil (in the ratio 2:1), with native resistant starch. The observed excessive hardening effect was reduced by increasing the moisture of AC to 60%. The authors verified that at this moisture level the further replacement of up to 90% of fat with starch allowed to obtain a 2% fat AC with hardness and cohesion values amenable to slicing, shredding and moulding. This research demonstrated that a high moisture content (60%) plasticized AC matrix allowing the cheese to melt even if it contained only 2% fat.” Revised lines 424–430.

15. **When discussion ‘functionality’ authors are discussing ‘nutritional value’ too!!**
    AU: We thank the Reviewer and in Line 174 of the original manuscript we deleted “nutritional properties”.

16. **Line 188 – Author is referring to pH of ~ 6.7 – can it be of analogue cheese?? Specify if such pH was being discussed for the cheese milk. In analogue cheese making, milk is never used!!**
    AU: We thank the Reviewer for Her/His comment.
    We modified the text as follows: “Anyway, acid CN is reported to hydrate better than rennet CN when converted to sodium or calcium caseinate by alkalinization because its water binding capacity is enhanced (Guinee, 2011).” Revised lines 189–190.

17. **Line 194 – What is ‘faultless taste’ – Do not use unconventional wrong words**

AU: We removed this Ref.

18. Line 197 – Acid casein is prepared by precipitating milk at below its isoelectric point – how can such acid casein hydrate so well (as mentioned!!)

AU: We thank the Reviewer for Her/His specification. The Reviewer refers to authors using acid-CN as protein source. They did not report any problem of acid-CN solubilization when preparing AC (neither directly nor an extension of processing time). We suppose that, under the conditions they adopted, the use of disodium phosphate (0.8%) increasing the pH of the CN dispersion contributed to the solubilization of acid-CN.

19. Line 198 – Give example of appropriate mixtures of ES as recommended by the concerned author.

AU: We introduced the following sentence: "Typically, a mixture of phosphates (disodium phosphate) and citrates (trisodium citrate) in the ratio of 1:1 is used in AC manufacture (El-Bakry et al., 2011b).". Revised lines 314–315.

20. Line 204 – Stringiness and Stretchability literally mean similar aspect.

AU: We thank the Reviewer for Her/His remark. Stringiness or stretchability means the ability of heated cheese to form strings and/or sheets when extended (Fox et al., 2017 – Chapter 18). We deleted the term stringiness. (revised line 197).

21. Use abbreviation MCA for Mozzarella cheese analogue

AU: We introduced the abbreviation in the text. Revised line 194.

22. Line 220 - Replacing which protein source and in which cheese analogue? What was the objective in replacing such protein source with dried UF retentate? You are reviewing paper – give critic on such aspect

AU: We preferred to remove this Ref..

Actually, this is a study of Abou El Nour, Scheurer, and Buchheim (1996) who investigated on the use of a casein-whey protein co-precipitate. The authors partially replaced (up to 50%) rennet CN with above protein derivative in AC formulation to obtain a block-type processed cheese analogue. As a consequence, cheese firmness increased and flowability of the melted product decreased to an extent that increased with the level of substitution. This negative functionality (i.e. the adverse effect on meltability) was partly associated to the uncontrolled heat-induced denaturation and aggregation of whey proteins and the subsequent formation of large aggregates of whey protein-casein, which affected the size/gelation capacity of the co-precipitate.

23. Line 224 - MPC had how much protein content –it ranges from 45-85% protein

AU: We preferred to remove also this Ref.. We introduced more details on a recent paper of Dhanraj et al. (2017) on the use of WPC as rennet CN replacer.

“Dhanraj et al. (2017) studied the influence of WPC as partial rennet CN replacer in the formulation of MCA used as pizza topping. Blends of rennet CN:WPC (68% protein) at ratios 95:15, 90:10, 95:5 were evaluated in terms of baking and sensory properties. MCA samples of different protein blends showed only slight differences in terms of shredding, meltability (Schrieber test), stretch test (Fork test) and total sensory scores (hedonic tests evaluating appearance, flavor, melting, and chewiness attributes). The sample with protein blend at CN:WPC ratio 90:10 was slightly preferred over the other blends.” Revised lines 226–232.

24. **Line 226 – Which type of WP was used – WPC or WPI?**

    AU: We specified as follows:

    We preferred to remove this Ref. for the sake of conciseness of the manuscript. We modified the text as follows: “Whey proteins (WP) derivatives (typically WPC), as the alternative to CN for nutritional and economic reasons, exert complex effects on the properties of AC.”. Revised lines 219–221.

25. **Line 259 - Inform the readers of limitations of CN in cheese system**

    AU: We modified the text as follows: “… (e.g. limited hydration capacity and solubility in water) …”. Revised lines 250−251.

26. **Line 268 -Specify when fat is in crystalline form in cheese and even in emulsified state as a result of ES action**

    AU: We specified as follows: “At room temperature, about 15% of the milkfat should still be a solid forming an exterior crystalline layer, while the oil in the interior of the fat globules. The solid component consists of a network of triglyceride crystals connected by Van der Waals forces of attraction. Following ES addition, shearing and heating actions during AC manufacture, the rupture of these bonds takes place, forming melted oil droplets of variable diameter, which are emulsified by the protein (Lobato-Calleros, Vernon-Carter, & Hornelas-Uribe, 1998).” Revised lines 261−266.

27. **Line 274 – Butter Oil is an inferior quality compared to AMF, when you mentioned AMF, BO need not be mentioned! (Check Codex standard for AMF vis a vis BO)**

    AU: We deleted butter oil.

28. **Line 298 – Mentioning type of vegetable fat and its melting point adjusted through hydrogenation of fat or by Interesterification - No mention of such aspect even under heading of ‘FAT’**

    AU: We introduced a new paragraph, as follows: “To elevate the melting point of a vegetable oil to a level similar to that of milk fat (32−34 °C), the former is required to be partly hydrogenated or suitably modified (interesterification). Cunha, Grimaldi, Alcântara, and Viotto (2013) showed that partially hydrogenated soybean fat (melting point of 36 °C) in substitution to butter oil (melting point of 34 °C) resulted in a spreadable analogue cheese with lower size of fat globules and greater hardness. Lobato-Calleros et al. (1998) found that AC formulations containing soybean fat (melting of point of 35−38 °C) had higher hardness than the AC made with 100% butterfat (melting point of 32−34 °C).” Revised lines 284−290.

29. **Line 314 - Increasing pH and buffering pH are two different parameters – do not club them together!**
AU: We modified the sentence as follows: "…,increase of pH value, stabilization (buffering) of pH level, ….". Revised lines 317−318.

30. **Line 321 – Kapoor and Metzger - Delete this contradicting statement to your earlier mentioned statement on ES action.**
AU: We deleted this statement.

31. **Line 335 - Name the ES used; conventional ES used was which combination?? How was such reduction in ES possible – given reasoning for the same!**
AU: We introduced a new paragraph. "El-Bakry, Duggan, O'Riordan, and O'Sullivan (2010) investigated the impact of decreasing sodium in AC, through reduction of ES concentration in the formulation from a standard level of 1.4% up to about 0.8%. In the standard AC the ES mixture consisted of trisodium citrate and disodium phosphate at a ratio of 2.16:1. Over the range of ES reductions, a progressive increase in processing time and in the mixing energy needed to emulsify the fat phase. As a consequence, a decrease of fat globule diameter was observed. The prolonged mixing during manufacture increased hardness and decreased the heat-induced flowability. Only a ES reduction of up to 20% was judged feasible, being above AC functionalities only slightly altered and processing times increased by only 25 %." Revised lines 353–360.

32. **Line 349 – Which type of CN was used – reader would like to know.**
AU: We thank the Reviewer. We corrected the sentence as follows: "Native starch is added to AC formulations at levels of 2–4% to substitute ~ 10–15% rennet CN for its relatively low cost, availability and benefits (Considine et al., 2011; Guinee, 2011)." Revised lines 391–392.

33. **Line 363 – specify the amylase content in the starch used by workers.**
AU: We modified the sentence, specifying the amylose content, as follows: "Starches with high amylose content (25% maize, 28% wheat) resulted in reduced flowability …". Revised lines 412–413.

34. **Line 378 - Cheese analogue cannot be obtained without use of cheese flavour – you wrote lack of flavour is incorrect.**
AU: We removed this sentence.

35. **Line 415 - Hydrocolloids seldom exerts emulsifying property at all, exceptions being Gum acacia or CMC! (Were these used by workers, who observed emulsification property exerted by hydrocolloids??)**
AU: We thank the Reviewer for Her/His detailed correction. We deleted “emulsifying”. Anyway, basil seed gum was reported to possess emulsifying properties. In a subsequent paragraph we reported “Hosseini-Parvar et al. (2015) explored the impact of basil seed gum (a surface-active polysaccharide-based hydrocolloid) on functional properties of AC. Analogue cheeses (52% moisture, 30% soy oil contents) were manufactured at different levels of proteins (6–10%), water (56–52%) and basil seed gum (0–1%). Increasing levels of added hydrocolloid in the formulations with the same protein content contributed to oil emulsification.” Revised lines 499−503.

36. **Line 425 – Which protein was AC based on - in which WP was also used**
AU: We specified type of CN used. “Acid CN-based processed cheese analogues (11.5% acid CN in the formulation) obtained with addition of WPI (0.5% in the formulation) and different hydrocolloids blends (up to 0.21% in the formulation) showed increased hardness and decreased adhesiveness (Gustaw & Mleko, 2007).”. Revised lines 489−492.

37. **Line 453 – Though NaCl can serve as flavour enhancer, salt is always added in cheese or cheese analogue formulation for the subtle salty taste and for preservation action.**
AU: We thank the Reviewer and introduced investigations on NaCl both in the section Preservatives (Khanipour et al., 2016) and Miscellaneous food additives (El-Bakry, Beninati, Duggan, O'Riordan, & O'Sullivan, 2011d; Gustaw and Mleko 2007).

38 **Line 466 – 4 type of acid mentioned – Rate of addition is only about 0.2% (it would vary based on the type of acid used, even type of ES salt will change the pH – some ES are alkaline, some are basic salts). Similarly for vitamin or mineral addition (line 484)**
AU: Acids (typically citric or lactic acids) are used up to levels such that the pH of the finished product reach the desired level. The mean level of acidifying agents reported in literature is 0.40% for MCA (Fox et al., 2017, page 617). We revised the text introducing a range of values, as follows: “Food-grade organic acids are used at concentrations of ~ 0.2–1%, as a function of the type of acid (see also Table 1). Also types of ES, having different pH-buffering capacity, affect subsequently the concentration of acidulants added in the formulation”. Revised lines 520–522.

For colors we wrote: "Colors included in AC formulation (annatto, paprika, artificial colors) are optional ingredients and typically are added at concentrations of 0.04% on average (Guinee, 2007)." Revised lines 599−600.

For other minor compounds, i.e. vitamin and mineral preparations we reported "Mineral and vitamin preparations introduced as nutritional supplements (at mean rates of 0–0.5%) (Chandan & Kapoor, 2011), include magnesium oxide, zinc oxide, iron, vitamin A, palmitate, riboflavin, thiamine and folic acid (Guinee, 2011)." Revised lines 600−603.

For the interest of readers we quoted the latest report of FAO/WHO Codex Stan 192-1995 (2016) on food additives. This document reports the maximum levels of some sweeteners, colors and preservatives permitted in AC. Really, sweeteners are hardly used in AC manufacture. Anyway, in this document the maximum amounts permitted in AC for this class of additives ranges from 33 ppm for neotame up to 1,000 ppm for aspartame. The range of maximum values permitted for colors is wider, ranging from 15 ppm for canthaxanthin up to 50,000 ppm for caramel.

39 Line 499 – What is 'remelting property'? Define it and use it in proper context, if true

AU: The authors reported the term "remelting properties" under the header "Manufacture of cheese analogues". Really, they referred to the melting properties of AC. We corrected into "melting properties". Revised line 617.

40 Line 505 and at someline later – Avoid using upward or lower adjustment of pH – Adjusting is to target pH only.

AU: We thank the Reviewer for Her/His correction. We detailed pH levels throughout the text. Please, see grey colored text in revised manuscript.

41 Discussion are missing on the following very important aspects

1. **Functional properties expected from the AC based on the cheese it is trying to mimic (e.g. stretch for Mozzarella/pizza cheese analogue)**
2. **Textural properties of AC vis a vis natural cheese**
3. **Microbiology of AC vis a vis natural cheese**
4. **Shelf life of AC and changes in functional properties of AC during its refrigerated storage**
5. **Cost aspects**

AU: We are grateful to the Reviewer for Her/His detailed suggestions to improve the quality of the manuscript. For the sake of conciseness (there is an editorial limit of 10,000 words for reviews), we inserted a new Section ("Functional properties of analogue cheeses *vs* natural cheeses and changes upon storage", revised lines 657–711) dealing with topics 1 to 4. Additional aspects on microbial stability were reported in revised lines 370–372. Cost aspects were inserted in revised lines 43–50.

42 **Not even one formulation of Analogue Cheese has been given in the entire manuscript. Authors can give Table containing formulations standardized by few research workers. Give formulation based on (i) Rennet casein, (ii) Na/Ca-caseinate, (iii) Caseinate + WP, etc.**
AU: We introduced a new table (revised Table 1) including examples of formulations for specific AC types.

43 **Deal with ingredients other than preservatives as next topic – Miscellaneous food additives (colour, flavour, etc.), sweetening agents are hardly used in ACs (not heard of it)**
AU: We added the new Section, as suggested. Revised lines 540–603.

44 **The referred Reference text should be studied and its main matter should be included in the review i.e. type of casein used in preparing Analogue cheese, which ES was used at what rate? Even the type of cheese analogue made (to imitate which cheese variety) should be indicated clearly.**
AU: Throughout the text we introduced details on several investigations. Please, see grey colored revised text.

45 **References**
Cunha et al.(2010) Check name of Journal as written
AU: We corrected the name of Journal.

46 **Name of Journal should be in Italics (Duggan et al.(2008)**
AU: We modified in Italics.

47 **El-Bakry et al. (2011b – check Ref. with 2011a) (For same year, volume number cannot be different for same Journal – 2011 year – 102 and in former Ref. 103?? Not possible)**
AU: We corrected Refs.

48 **Provide page numbers for Chapters quoted from Books e.g. Fox et al. (2017a), etc.**
AU: We introduced page numbers.

49 **Tamplin (2016) Name of bacteria to be in Italics**
AU: We changed the name in Italics.

50 **Look at sequencing of References e.g. Mohamed and Shalaby (2016)**
AU: We removed these Ref.

51 **Noronha et al. (2008) was at lower pages, which has tobe brought first writing 2008a – Next Ref. (2008a) will become (2008b), etc……. for 2008c, 2008d and 2008e.**

AU: We corrected the order of Refs. Please, see revised manuscript.

52 OECD (2015) Write title of the paper quoted.

AU: This is a book. "OECD (2015). OECD-FAO Agricultural Outlook 2015-2024. Food and Agriculture Organization of the United Nations (pp. 21-59). Paris:OECD Publishing, (Chapter 1)."

53 Williams and Carter (2011) Write title of the paper referred.

AU: We inserted the book title. "Williams, E. M., & Carter, S. J. (2011). *The A-Z encyclopedia of food controversies and the law*. Santa Barbara: Greenwood ABC-CLIO LLC."

54 Table 1 needs to be incorporated as textual matter only in manuscript.

AU: We deleted Table 1 and incorporated the content in manuscript. Revised lines (43–50).

55 Table 2 is too large (5 pages continued). Several aspects included in the Table is already mentioned in the text (Repetitions are not warranted at all). Within this Table, aspects with similar topic (i.e. caseinate substitution with whey proteins) should be clubbed together and dealt in table. Choose only selected work on AC and make Table preferably in 1 page only.

AU: We are grateful to the Reviewer for the comment.

Anyway, in the text of the revised manuscript we introduced several additional details on some investigations. Thus, we preferred to modify the format of the table, removing the column dealing with results. We introduced a revised Table 2 entitled "Recent research topics in analogue cheese formulations and manufacture". In column 1 we reported the most recent topics of interest and in column 2 we quoted the corresponding Refs.

56 Remove brackets for the References quoted in Table 2, since they are in last column only.

AU: We removed brackets.

57 Particulars written in this Table for Lee et al. (2012) is already mentioned in the text. No need to repeat the same under Table 2.

AU: We thank the Reviewer. We removed Lee et al. (2013) from revised Table 2 and introduced it in the text.

58 Fig. 2

Name the dry ingredients

Name the type of oil used and desired melting point too

Specify the desired pH of cheese analogue (for a specific cheese type it is supposed to imitate)

AU: We thank the Reviewer for the suggested improvements. To keep the style of figure, we preferred to introduce all suggested modifications in the revised text. Please, see revised lines 612−613 for dry ingredients, revised lines 622−623 for type of oils, revised lines 284, 285, 287, 290, 466 for melting points, revised lines 514−517 for pH values.

Response to Reviewer #2
Ref: TIFS_2017_556
Title: STATUS AND DEVELOPMENTS IN ANALOGUE CHEESE FORMULATIONS
Journal: Trends in Food Science & Technology

To simplify reading, the original points raised by the Reviewer are in black bold and our answers are in black typeface after the initials AU:.
Changes in the revised manuscript are highlighted in grey.

L84: what is CN? Write full name first, then use abbreviation.
AU: We thank the Reviewer and wrote full name in revised line 52.

L519: what are the plants? Not clear
AU: We specified this point and introduced more details on the subject.
"Different types of in-batch cookers with specific design and operating conditions are available for AC processing. Such plants are generally adopted in the manufacture of batches of 2–4 kg. The most common type has single/twin-screw augers with agitation speeds ranging from 100 to 200 rpm (Blentech cooker). This reduced shear action promotes a low fat dispersion, resulting in large fat particles (diameter 5–25 μm). Consequently, the final AC upon baking is characterized by the desired performances when used as pizza topping (i.e. sufficient degree of oiling off and limited dehydration) (Fox et al., 2017a). An alternative to the twin-screw cooker is the single-blade cooker (Stephan cooker, generally adopted for processed cheeses), which is characterized by higher speeds (up to 1,500 rpm). Noronha, O'Riordan, and O'Sullivan (2008d) compared the performances of cheese system in cheese plants and focused on the effect of different agitation speeds (100 to 200 rpm with Blentech and 750 to 1,500 rpm with Stephan) at a constant holding time (~ 2 min) at 80 °C. The authors observed that increasing the speed of agitation in the Blentech cooker slightly affected hardness and meltability values of AC, whereas increasing the blade speed of the Stephan cooker caused a decrease in the size of fat globules with a significant increase in the hardness and a decrease of the meltability of AC. They concluded that functional properties (texture, melting and color) of the end-product were acceptable also in single-blade cooker when operating at moderate speeds (750 rpm)." Revised lines 630–645.

L548: this is a review article, not research.
AU: We modified as follows: "The preparation of this article did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors."

Paper can also include brief info on sensory properties of these cheeses.
AU: We thank the Reviewer for Her/His suggestion. We detailed results on sensory properties of AC throughout the text. Revised lines 198–205, 212–218; 226–232; 424–434; 554–564.

Response to Reviewer #3

Ref: TIFS_2017_556
Title: STATUS AND DEVELOPMENTS IN ANALOGUE CHEESE FORMULATIONS
Journal: Trends in Food Science & Technology

To simplify reading, the original points raised by the Reviewer are in black bold and our answers are in black typeface after the initials AU:.

Overall Comments
Analogue cheese (AC) is a cheese-like imitation that is targeted specifically towards the low-cost end of the 'cheese' market. As pressure continues to drive mass food process downwards, manufacturers of AC seek new approaches to minimize protein content and maximize the content of water and cheap ingredients. Consequently, a review of the literature and the exposure of new approaches is of interest. The current manuscript systematically reviewed the literature on the different ingredients and processing conditions. However, the writing style is generally quite 'loose', resulting in, many cases, little detail or proper critiquing of the cited studies. A scientific review requires such analysis to chart the present state of the art and propose new approaches to future innovation. Hence, substantial 'tightening' of the syntax and more precision are required in this respect. It also needs to project to potential future scope for AC.
Based on these comments, and the specific comments below, I recommend major revision and resubmission for further review.
AU: We are really grateful to the Reviewer for several amendments and suggestions to improve the scientific quality and the writing style of the manuscript. To answer Reviewers' comments we introduced a new section ("Functional properties of analogue cheeses *vs* natural cheeses and changes upon storage"). As suggested, we focused on more relevant works. Changes in the revised manuscript are highlighted in grey.

Specific Comments

1. **Lines 71-72." The US is the major producer (~ 3 × 10^6 t per year)".**
   Q. This quantity (3 million tonnes per year) is very large and would appear to be incorrect?
   AU: We thank the Reviewer for Her/His amendment and corrected the text "(~ 3 × 10^5 t per year)". This amount was quoted by Fox et al. (2017a).

2. **Lines 116-117. Water is necessarily used to dissolve ES, to hydrate proteins and to solubilize other ingredients. It behaves both as gelling and emulsifying agent.**

AU: These lines were reported in the manuscript. There is no comment. We removed the sentence "It behaves both as gelling and emulsifying agent". Really, rehydrated protein behaves as a water-binding and emulsifying agent.

3. **Lines 20-221. "Bachmann (2001) reported that up to a replacement of 40% dried ultrafiltration retentate of skim milk the resulting AC showed positive organoleptic and texture properties."**
   Q. It is unclear what this sentence means: replacement of "40% dried ultrafiltration retentate of skim milk" with what? Re-write.
   AU: We preferred to remove this Ref..
   Actually, this is a study of Abou El Nour, Scheurer, and Buchheim (1996) who investigated on the use of a casein-whey protein co-precipitate. The authors partially replaced (up to 50%) rennet CN with above protein derivative in AC formulation to obtain a block-type processed cheese analogue. As a consequence, cheese firmness increased and flowability of the melted product decreased to an extent that increased with the level of substitution. This negative functionality (i.e. the adverse effect on meltability) was partly associated to the uncontrolled heat-induced denaturation and aggregation of whey proteins and the subsequent formation of large aggregates of whey protein-casein, which affected the size/gelation capacity of the co-precipitate.

4. **Lines 224-225. "Moreover, a partial replacement of rennet CN with milk protein concentrate was effective in terms of texture and sliceability of spread-type AC."**
   Q. Is sliceability of spread-type AC ok. It is not expected that sliceability would be an attribute of a spread-type product?
   AU: We preferred to remove also this ref.. We introduced more details on a recent paper (Dhanraj et al., 2017) on the effect of using WPC as rennet CN replacer. "Dhanraj et al. (2017) studied the influence of WPC as partial rennet CN replacer in the formulation of MCA used as pizza topping. Blends of rennet CN:WPC (68% protein) at ratios 95:15, 90:10, 95:5 were evaluated in terms of baking and sensory properties. MCA samples of different protein blends showed only slight differences in terms of shredding, meltability (Schrieber test), stretch test (Fork test) and total sensory scores (hedonic tests evaluating appearance, flavor, melting, and chewiness attributes). The sample with protein blend at CN:WPC ratio 90:10 was slightly preferred over the other blends.". Revised lines 226–232.

5. **Lines 245-247. "As expected, firmness resulted sensitively higher in comparison to AC obtained solely from acid CN when pH was set in the range 4.5–5.0, whereas meltability was lower in the pH range 5.0–7.0.".**
   Q. It is not clear what is meant by "sensitively higher"? re-write.
   I do not have the paper the paper by Sołowiej, Mleko & Gustaw (2008) at hand while reviewing this manuscript – hence, I do not know the context of the

experiment. However, acid casein is essentially insoluble at pH 4.5 – 5.0; consequently, making an analogue from acid casein at this pH does not make sense. So yes, of course if the protein is insoluble/not hydrated it is small wonder that the analogue from acid casein at pH 4.5 – 5.0 is less firm than that with the WPC35. Hydration of any protein is a prerequisite for functional expression.

When reviewing the literature on the topic, as the authors are doing in the current review, the context should be given and if the results are meaningful, then it is acceptable to state the findings. Otherwise, if the results are not, then I authors should give an informative critique as to why the results are not, or else not include them in the review. What was the % total protein content, the % casein, the % WPC and % fat in the analogue of Sołowiej, Mleko & Gustaw (2008)

AU: We are really grateful to the Reviewer and we completely agree on our poor writing style. We preferred to remove also the paragraph dealing with the paper by Sołowiej, Mleko & Gustaw (2008). We introduced more details on a recent paper we quoted in the original manuscript (Sołowiej, Cheung, & Li-Chan, 2014). Revised lines 232–239.

These authors studied functionalities of AC from different types and combinations of protein sources, i.e.

rennet-CN (11%, 12% or 13%),

acid-CN (11%, 12% or 13%),

acid-CN+WPI/WPC (10% + 1%, 10% + 2%, 10% + 3%, respectively),

rennet-CN+WPI/WPC (10% + 1%, 10% + 2%, 10% + 3%, respectively).

In relation to the solubilization of acid-CN we report herewith the AC manufacture protocol. Dry protein ingredients were dispersed in distilled water at 21°C using a magnetic stirrer (300 rpm), then mixed for 2 min at 10,000 rpm with anhydrous butterfat (previously melted at 40 °C). After adding disodium phosphate (0.8%), the mixture was heated at 80 °C and mixed at 10,000 rpm for 10 min. Then the pH value was adjusted to 6.2, using 40% citric acid or 2 M sodium hydroxide. We suppose that the addition of disodium phosphate resulting in an increase in pH value of the acid-CN dispersion, contributed to the solubilization of CN.

6. **Lines 251-252. "Anyway, cohesiveness decreased with concentration of the added whey derivative."**

   Q. Again here, a bit of context is required. From a practical context (say for some manufacturer of AC reading this current article), what is the relevance of reduced cohesiveness in terms of AC? A review should give the state of the art, and if possible attempt to analyse the literature for its progress of the state of the art.

   AU: We thank again the Reviewer for Her/His amendment. Anyway, following your previous suggestion, we preferred to remove this Ref. (Sołowiej, Mleko, Gustaw, & Udeh, 2010). We focused on AC varieties using rennet CN rather than those using acid CN, being the former the most used source of protein in AC.

7. **Lines 252-253. "An increase in the concentration of acid CN or whey products entailed a decrease in the meltability of AC, which still remained very good" Appears somewhat contradictory. What does very good mean? Does it mean that increasing the concentration of acid CN or whey products (demineralized whey powder, WPC35) to ? % in AC containing ? % rennet casein exceeded a certain meltability (e.g. ? %), as determined using some (e.g., Schreiber?) method? As per comment 4, for the review to be meaningful, more context and details of the cited studies need to be given.**

AU: We removed also this Ref.. We preferred to focus on the results of Lee, Huss, Klostermeyer, & Anema (2013) on the effects of inclusion of WP derivatives at increasing rates of WP denaturation in AC. "The addition of WPC (80% protein) solutions at different degrees of denaturation (by heating at temperatures from 67 °C to 85 °C for holding times of 150 s in a scraped-surface exchanger) in the formulation affected the rheology and microstructure of rennet CN-based spreadable AC (Lee, Huss, Klostermeyer, & Anema, 2013). Degrees of denaturation in AC with microparticulated aggregates ranged from 40% to 100%. Higher levels of WP denaturation in AC caused a decrease in hardness and an increase in the meltability of the resultant spreadable AC compared to samples with unheated WPC solution. This effect was attributed to the incorporation of denatured WP as relatively large particles that did not contribute to the matrix structure, thus disrupting the AC network. In contrast, following the addition of native WP, the manufacturing conditions of AC promoted a only partial denaturation (~ 40%) of WP resulting in their subsequent incorporation into the matrix, producing a stronger structure.". Revised lines 240−249.

8. **Lines 255. "The resulting product resembled the traditional counterpart". What was the traditional product made with? Rennet casein and milk fat?**

   AU: We thank the Reviewer for the comment. We preferred to remove the Ref. considering it not worth of relevance in this context. This whey cheese was made from whey of hard or semi-hard cheeses.

9. **Line 259. "some functionality limitations of CN".**

   Q. What are the functionality limitations of casein? And is this rennet casein, acid casein, sodium caseinate, calcium caseinate, or all of these?

   AU: We introduced some functional limitations. "… (e.g. limited hydration capacity and solubility in water). Revised line 250−251.

10. **Lines 287-290. "Generally, the replacement of milk fat by vegetable oils results in softer AC (Budiman, Stroshine, & Campanella, 2000; Shabani, Sarfarazi, Mirzaei, & Jafari, 2016), while the adoption of fats results in harder or similar consistency in comparison to those with butter (Cunha, Dias, & Viotto, 2010)".**

    Q. What does "adoption" of fats mean? Hydrogenation?

AU: We thank the Reviewer and modified the text as follows: “Generally, the total or partial replacement of milk fat by vegetable oils (up to 50%) (liquid at room temperature) resulted in softer AC (Budiman, et al., 2000; Cunha, Dias, & Viotto, 2010; Shabani, Sarfarazi, Mirzaei, & Jafari, 2016), while the use in the formulation of vegetable fats resulted in harder or similar consistency AC in comparison to that of butter. To elevate the melting point of a vegetable oil to a level similar to that of milk fat (32−34 °C), the former is required to be partly hydrogenated or suitably modified (interesterification). Cunha, Grimaldi, Alcântara, and Viotto (2013) showed that partially hydrogenated soybean fat (melting point of 36 °C) in substitution to butter oil (melting point of 34 °C) resulted in a spreadable analogue cheese with lower size of fat globules and greater hardness. Lobato-Calleros et al. (1998) found that AC formulations containing soybean fat (melting of point of 35−38 °C) had higher hardness than the AC made with 100% butterfat (melting point of 32−34 °C).” Revised lines 280−290.

11. **Lines 295-296. “An item of interest for manufacturers since the 1990s has been the production of new cheese products low in fat. Reducing the fat content of AC modifies its performances”**
    Q. How, in what way, does fat reduction modify performance of AC and, basically, why?
    AU: We thank the Reviewer. We modified the text as follows: “Fat reduction causes an increase in the protein network and a decrease in lubrication upon melting, resulting in a hard and crumbly texture which is undesirable from a sensory perspective. The adverse effects of a high concentration of protein on the properties of low-fat AC may be attenuated by modifications of the manufacturing process to reduce the levels of calcium-to-CN ratio and by increasing the levels of moisture. Increased hydration has a plasticizing effect (Noronha 2007) (See also the section Starch).”. Revised lines 301−306.

12. **Lines 312-313. “For instance, by increasing phosphate:citrate ratio, the matrix could be softened to obtain AC with reduced fat content”**
    Q. What was (or what do the authors believe) was the mechanism by which an increase the phosphate:citrate ratio increased the softness of AC? What is related to a change in pH. The review should try to analyse trends such as this.
    AU: We are grateful to the Reviewer for Her/His remark. We introduced details to explain how the ES ratio affects hardness in AC. “El-Bakry et al. (2011b) verified the influence of different ratios of a disodium-phosphate:trisodium-citrate blend, from 0:1 to 1:0 in the manufacture of a 50% moisture rennet CN-based AC. The authors verified that changes from the standard ES ratio (1:1) modified the hardness of the final product. By increasing phosphate:citrate ratio from 1:1 up to 1:0, the mean fat globule size in AC increased from 20 µm to 31.5 µm and the cheese matrix was softened. Under these conditions, protein-protein interactions were supposed to be less disrupted due to low calcium solubilization. The consequent lower hydrated CN resulted in reduced fat emulsification, ultimately leading to a less plasticized matrix. Differently, the cheese made with citrate as a sole ES had the smallest fat globule size (7.6 µm)

and highest hardness. Thus, altering the ratio in the ES blend may allow tailoring AC properties for specific end uses. For instance, cheese can be softened by increasing disodium-phosphate:trisodium-citrate ratio, e.g. in case of reduced fat AC." Revised lines 324−334.

13. **Lines 345-346. "Over this period, a reduction in quality was observed." Q. How did the reduction in quality manifest? And was this reduction observed in cheeses made with the control AC and with AC containing varying degrees of substitution of sodium ES with potassium ES?**
    AU: We removed the paragraph about the paper of Schatz et al., 2014 and preferred to better describe results obtained by El-Bakry et al. (2011a) on the same topic.
    "A reduction of sodium content was achievable also by the replacement of sodium ES blend (disodium phosphate and trisodium citrate, at ratio 0.46:1.0) with their potassium equivalents (El-Bakry et al., 2011a) in a 48% moisture rennet-based AC. This substitution led to better protein hydration which required less mixing energy of AC ingredients. Adding potassium-ES (instead of sodium-ES) resulted in a slight increase in pH value (from 6.0 to 6.3) due to less potassium binding (with respect to sodium) to CN and to a less displacement effect of hydrogen ions of CN. The increased pH was hypothesized to increase electrostatic repulsion between the CN molecules, thus improving protein hydration and, as a consequence better fat emulsification. Differences were observed during refrigerated storage in functional properties. The increases in pH value and fat globule size promoted slight decrease in hardness, a less cohesive structure and slight increase in flowability and adhesiveness. The increase in pH value of AC obtained with potassium ES was hypothesized as the cause of their reduced microbial stability during 12 weeks storage (at 4 °C) in comparison to the sodium equivalents. The trial was carried out at laboratory-scale and thus under hygienic conditions not as strict as in industrial processing (i.e. under controlled air flow and using aseptic packaging). After manufacture, the samples with sodium ES (pH 6.0) showed a total viable count of ~ 10^1 ufc g^{-1}, which slightly increases up to 10^2 cfu g^{-1} throughout the storage period. The substitution of the sodium salts by their potassium equivalents led to substantial higher counts (up to10^4 cfu g^{-1}). In either formulations no mould or yeast growth was detected.". Revised lines 360–377.

14. **Lines 359-361. " Following the addition of native starch, oil droplet sizes were smaller than in starch-free AC (Mounsey & O'Riordan, 2001) suggesting a more severe fat emulsification."**
    Q. The suggestion (higher starch, greater fat emulsification) is somewhat difficult to understand, considering that starch would make less water available for the hydration of casein which is the emulsifying agent. Starch itself is highly hydrophilic, is not amphipathic, and is likely to emulsify fat like protein does?
    AU: We thank the Reviewer for the comment. We corrected the syntax. Starch did not emulsify oil. The authors reported that during the manufacture of AC the oil of starch-containing products was emulsified more rapidly compared to the control product. Microscopic images of

the AC showed a uniform distribution of spherical fat droplets in a smooth protein matrix. Starch behaved like a filler in the early, low-temperature stages of manufacture, leading to increased availability of water to hydrate the CN and increase its fat emulsifying properties. We modified the text as follows: "The partial replacement (15%) of rennet CN with wheat, maize, waxy-maize or rice starches resulted in AC with fat globule diameters (2–10 µm) smaller than the starch-free control, thus indicating more extensive fat emulsification (Mounsey & O'Riordan, 2001). The authors observed that during AC manufacture the oil of starch-containing AC was emulsified more rapidly than the control. The starch likely behaved as a filler in the early, low-temperature stages of AC manufacture, leading to increased availability of water to hydrate the CN, and subsequently improved fat emulsification. An important impact of partial substitution of rennet CN with starch was the reduction in AC meltability in the order maize > waxy-maize > wheat > rice (Mounsey & O'Riordan, 2001, 2008d). This effect was attributed to different factors such as fat globule size and the disruption of protein matrix by swollen starch granules. The latter favoring the immobilization of water by the granules may have resulted in the dehydration of the protein matrix, which led to increased hydrophobic protein-protein interactions and ultimately poorer meltability.". Revised lines 395−406.

15. **Lines 365-366. "Analogue cheeses with pre-gelatinized starches showed poorer fat emulsification resulting softer, less cohesive and with reduced meltability (Mounsey & O'Riordan, 2008b)."**
    Q. The syntax (sentence structure) is somewhat unclear. Do the authors mean 'Analogue cheeses with pre-gelatinized starches showed poorer fat emulsification resulting in softer, less cohesive AC that had reduced meltability (Mounsey & O'Riordan, 2008b)'
    AU: We appreciate the Reviewer's correction and modified the text as suggested. Revised lines 418−420.

16. **Lines 370-371. "Trivedi et al. (2008) verified that incorporation of starch and low levels of CN replacement in the formulation of AC caused satisfactory rheological properties at lower ingredient cost."**
    Q. What are low levels (%) of CN replacement?
    AU: We thank the Reviewer. We preferred to remove this quotation from the text. We quoted this Ref. in Table 2.

17. **Lines 380-381. "Increasing the starch content of AC reflected in an increase in hardness."**
    Q. Is this resistant starch, and to which study doe the statement refer to: Noronha et al., 2008a?
    AU: We are grateful to Reviewer to underline the ambiguous writing style.

We corrected the text as follows: "Resistant starch was investigated as a source of dietary fiber to provide AC with the image of a health promoting and more nutritious food. Noronha et al. (2007) in an AC with 52% moisture and 12% fat contents replaced about 50% fat, consisting of hydrogenated palm oil and rapeseed oil (in the ratio 2:1), with native resistant starch. The observed excessive hardening effect was reduced by increasing the moisture of AC to 60%. The authors verified that at this moisture level the further replacement of up to 90% of fat with starch allowed to obtain a 2% fat AC with hardness and cohesion values amenable to slicing, shredding and moulding. This research demonstrated that a high moisture content (60%) plasticized AC matrix allowing the cheese to melt even if it contained only 2% fat. After sensory analysis by ranking tests, this high-moisture low-fat AC lost its grainy texture and panellists perceived it as smoother and firmer in comparison to samples where up to 85% fat content was replaced. The improved mouthfeel was attributed to the fact that more abundant and smaller starch particles in the 2% fat cheese may not have absorbed as much water as particles in the cheeses containing lower levels of starch." Revised lines 423–434.

18. **Lines 387-388. "The expansion process is mainly influenced by moisture content, whereas resistant starch does not swell during microwaving." Q. 'Resistant starch does not swell during microwaving': does not swell well compared to what other type of starch?**
    AU: The starch used in this study (high amylose corn starch, Hi-Maize 260) has less capacity to absorb water compared to other common corn starches or native resistant starch such as Novelose 330. The authors used ungelatinised, high amylose corn-starch which binds little water. This starch has a high gelatinisation temperature (99.7 °C). Although during microwave heating temperatures as high as 160 °C were reached, no swelling was observed. Really, granules expanded to some degree, but they largely maintained their structure and birifrangence. Starch granules did not swell well during microwave heating due to the limited water near the granules as most of it quickly evaporated.
    We modified the text as follows: "The resistant starch used, i.e. high amylose corn starch has a low water-holding capacity and does not swell during microwaving due to the insufficient water in the vicinity of the granules as most of it evaporates quickly due to the rapid nature of microwave heating.". Revised lines 443–446).

19. **Lines 390-392. "Moisture exerts a primary role also during the storage of AC prior to microwave heating by promoting an increase in the degree of expansion during heating." Q. Unclear. What is the 'primary role' that moisture causes during storage, which promotes the 'degree of expansion' during heating?**
    AU: We modified the text as follows: "The authors observed (by nuclear magnetic resonance relaxometry) that tightly bound water decreased during AC cold storage (up to 9 days). Such phenomenon was attributed to an increase in water mobility. The more mobile water increased the plasticization of the AC matrix making it more stretchable under pressure during microwaving. After 9 days storage the volumetric expansion of 60% moisture AC increased about 4-fold in comparison to 45% moisture AC." (revised lines 446–451).

20. **Lines 401-403. "Hennelly et al. (2006) reported that the form of incorporation of inulin (gel or solution) in AC formulation, to replace up to 63% fat, had similar effects in terms of microstructure and melting behavior of AC."**
    Q. Unclear. What was the 'form of incorporation of the inulin', and 'had similar effects in terms of microstructure and melting behavior of AC' compared to what?
    AU: We selected results of the investigation and modified the text as follows: "The simplest method of incorporation in AC would be as dry powder, but in this way its inclusion would lead to hard lumps and free water. To improve the distribution of inulin in AC, Hennelly et al. (2006) studied the effect of incorporating inulin either as a heated solution (80 °C) or as a shear-induced gel to replace 63% hydrogenated palm oil in a 54% moisture AC. The fat content was 11.0% in AC samples with 3.4% inulin, while in the control sample (inulin-free) was 21.7%. As a result of fat replacement, rennet CN content passed from 20.1% in the control sample to 23.8% in AC containing inulin. The inulin incorporation as a gel or as a solution had no significant effect in terms of AC microstructure and meltability in comparison to the control sample. The presence of inulin in AC caused a significant increase in hardness in comparison to inulin-free samples. Such result was apparently unexpected because hydrogenated palm oil is a hard fat (melting point 48 °C). Its substitution with a soft inulin gel or solution would be expected to result in a decrease in hardness. Being unchanged AC moisture level (54%), this virtual softening was more than offset by the increase of protein:fat and protein:moisture ratios of inulin samples with respect to control sample." Revised lines 457–469.

21. **Lines 403-405. "Besides, the presence of inulin in AC caused a significant increase in hardness (at equivalent moisture levels) in comparison to control AC with fat, whereas samples with different amounts in inulin (5% or 13.75%) were similar in hardness"**
    Q. Unclear. 'the presence of inulin in AC caused a significant increase in hardness (at equivalent moisture levels) in comparison to control AC with fat' – what level of inulin? Not 5% or 13.75%?
    The presence inulin at levels of 5% or 13.75% had similar hardness.
    AU: We thank the Reviewer for the comment. Please, se reply above.

22. **Line 407. "following partial replacement of fat by inulin in acid CN-based AC, reported textural, rheological and melting modifications".**
    Q. What kind and degree of modifications?
    AU: We selected results of the investigation and modified the text as follows: "In acid CN-based (10%) processed cheese analogues (13% protein, 30% fat contents) added with WP polymers (1–3%), Sołowiej et al. (2015) investigated the effect of the partial replacement of anhydrous milk fat (5–15%) with inulin (1–3%). Modifications in AC consisted in increased meltability (with 2–3% inulin) and decreased hardness (with 1% inulin) and adhesiveness (with 3% inulin) in comparison with full-fat (control) samples. The authors suggested that above effects in the CN-WP polymers-inulin/fat matrix could be exploited in low-fat processed

analogue cheeses to offset the undesired modifications of fat reduction.”. Revised lines 469–475.

23. **Line 419. Xanthan gum is anionic, as it contains glucuronic acid and some pyruvate ketal group.**
    AU: We thank the Reviewer and corrected the text.

24. **Lines 427-430. “Kappa- and λ-carrageenans resulted feasible replacers, but the final products were characterized by excessive hardness and reduced spreadability (Černíková et al. 2010) requiring further studies for the optimization of the formulation.”**
    Unclear. What does ‘resulted feasible replacers’ mean exactly?
    ‘And were characterized by excessive hardness and reduced spreadability’ – compared to what? The review needs to state this.
    AU: We preferred to remove this Ref.. The subject of the investigation were processed cheeses (Edam cheese-based) not AC.

25. **Lines 430-432. “Hosseini-Parvar et al. (2015) explored the impact of basil seed gum (a newcomer hydrocolloid) on functional properties of AC. The authors verified the viability to make AC with higher firmness and slightly lower meltability in comparison to the hydrocolloid-free counterpart.”**
    Newcomer refers to a person! Perhaps, a gum that has been recently (in the last decade?) introduced into modern food formulation.
    ‘The authors verified the viability to make AC with higher firmness and slightly lower meltability in comparison to the hydrocolloid-free counterpart’. Poorly written. The authors need to be categorical, for example: ‘In analogue cheeses with similar composition, AC containing ? % added basil gum was firmer, but less meltable than the control cheese’
    AU: We thank the Reviewer for the correction. We introduced more details on this research. “Analogue cheeses (52% moisture, 30% soy oil contents) were manufactured at different levels of proteins (6–10%), water (56–52%) and basil seed gum (0–1%). Increasing levels of added hydrocolloid in the formulations with the same protein content contributed to oil emulsification. By adding basil seed gum, it was possible to manufacture AC with higher firmness, but slightly lower meltability, and at lower cost owing to the lower protein and higher moisture contents. For instance, replacing 4% of the protein content (i.e. from 10% to 6%) with 0.1% basil seed gum and 4% water (from 52% to 56%) allowed to obtain an AC with slightly lower meltability than the sample with 10% protein (0% basil seed gum and 52% moisture contents). The rationale was the formation of a hydrocolloid network capable to strengthen the network formed by casein strands.” Revised lines 500–509.

26. **Line 435. ""Liu, Xu, and Guo (2008) investigated the role of pectin gel as fat mimetic on the physico-chemical parameters of low-fat AC. The pectin gel, linked with other ingredients, made AC more compact than pectin-free AC."'. Q. Investigated pectin? Pectin undergoes gelation to form a gel, depending on the type and conditions (pH, temperature, concentration). What type of pectin, high methoxy, low methoxy, amidated? "What does 'compact' mean here? Higher fracture stress, higher elasticity modulus?? As per comment 8, a scientific review should be precise, and give context context/details and objective analysis of cited studies.**

    AU: We deleted this Ref..

    Liu, Xu, and Guo (2008) used low-methoxylated pectin. "Pectin gel was prepared by mixing the pectin with water and interacted with calcium ion to form a weak-gel". The author provided no conditions on pectin gel preparation.

27. **Lines 442-443. "The use of little amounts of cheese in the formulation is a way to overcome this defect"**

    How much is little? 0.5%, 2%, 5% ??. Presumably, mature cheese?

    AU: Fox et al. (2017a) reported an amount of added cheese up to 50 mg/kg of final product. We did not find any reference quoting adding such low amounts of cheese. We suppose that mature cheese should be preferred to impart flavor.

28. **Lines 445-446. "Addition of sodium chloride in AC with WP and hydrocolloids modified textural properties (firmness increased and adhesiveness decreased) (Gustaw & Mleko, 2007).".**

    Again here, very loose writing style for scientific review: how much NaCl, what textural properties were modified, and how?

    AU: We detailed results reported by these authors. "Gustaw and Mleko (2007) investigated the influence of NaCl concentration (from 0.5 to 2% in the formulation) on texture properties of AC formulated with 11.5% acid CN, 0.5% WPI and 0.07–0.21 hydrocolloids blends (see also the section Hydrocolloids). Independently to the hydrocolloid blend used, increasing the NaCl concentration boosted linearly the effect of the increased concentration in hydrocolloids, i.e increased AC hardness and decreased adhesiveness. This phenomenon was explained by the influence of cations (sodium of NaCl) on the gelling behavior of polysaccharides." Revised lines 568–574.

29. **Line 457. "……NaCl AC indicated that reducing NaCl did…".**

    Q. reducing salt from what? To what? Did not affect what acceptability as measured by descriptive sensory analysis/hedonic analysis.

    AU: We detailed results reported by these authors.

    "In a further work on this topic, El-Bakry et al. (2011d) studied the effects of different NaCl concentrations (0−1.5%) in rennet CN-based AC (48% moisture) on physico-chemical properties, cheese manufacture and functionality of the final product. On a small-scale cooker

(Farinograph, 800 g of AC/batch), the authors observed that reducing NaCl from 1.5% up to 0% resulted in a progressive reduction in total mixing energy and total processing time, an increase in pH value (from 6.0 up to 6.5), an increase in water activity (from 0.975 up to 0.982), an increase in fat globule diameter (from 18 up to 29 µm), a significant decrease in hardness and a slight increase in flowability on melting. A similar trend of results was obtained using a pilot-scale cooker (Blentech, 4 Kg/batch). The higher pH value observed upon the reduction of NaCl was ascribed to the less displacement of hydrogen from the CN by sodium. This result reflected in higher net negative charges of CN molecules and higher electrostatic repulsions between them, leading to an increase in protein hydration. Shorter processing times, i.e. less shearing of the molten mass led to bigger fat globules. Both the increase of fat globule size and the increase in pH contributed to changes in post-manufacture cheese properties (hardness and flowability). Microbial count was 10^3 cfu g^{-1} after manufacture reaching ~ 10^4 cfu g^{-1} after 4 weeks (at 4 °C) independently of NaCl content. Anyway, at 0% NaCl microbial stability was reduced after 12 weeks (10^6 cfu g^{-1} while in AC with 1.5% NaCl remained at ~ 10^4 cfu g^{-1}). From time 0 to 4 weeks storage moulds and yeasts were absent in control AC (with 1.5% NaCl). Differently, the yeasts count after 2 weeks reached 10^3 cfu g^{-1}. Both pH and water activity increases were responsible of the reduced microbial stability. On the whole, the authors remarked the feasibility to reduce NaCl content in AC by adjusting of the final pH value with citric acid." Revised lines 574–593.

30. **Lines 480-482. "More recently, positive results were obtained by introducing vegetables and fruits in formulation to fortify AC with bioactive components (Mohamed & Shalaby, 2016; Mohamed, Samah, Shalaby, & Gafour, 2016)."**
    Q. Positive in what respect?
    AU: We deleted these Refs.. The authors manufactured cheeses using skim milk powder rennet coagulated to obtain a cheese base subsequently used in the manufacture of the "analogue" cheese.

31. **Line 511. "Shear rate affects the degree of emulsification of the cheese-like matrix."**
    Q. How, in what way? Surely, affects emulsification of the fat, not the matrix (which refers to the structure of the admixture of fat, protein, moisture and other ingredients).
    AU: We thank again the Reviewer for the amendment. We removed this statement. The effect of shear rate on the degree of fat emulsification was reported throughout the text.

32. **Line 538. "The upward potential of AC production".**
    Q. Refer to the published data or other evidence to support the statement, that analogue cheese production is growing.
    AU: Under conclusions header it is preferable to avoid the use of Refs. We introduced a Ref. supporting this statement in introduction. "An increase in the production of new varieties including AC, fulfilling the growing demand in developing dairy markets, is expected (PMFood & Dairy Consulting, 2014)." Revised lines 35–37.

33. **Conclusions: Need more emphasis on likely/possible developments that would improve quality (e.g., textural, sensory, nutritional), reduce cost, innovate new properties?**
AU: We tried to give more emphasis to conclusions. Please, see Conclusions Section.

Additional answers to Reviewer #1
Ref: TIFS_2017_556
Title: STATUS AND DEVELOPMENTS IN ANALOGUE CHEESE FORMULATIONS
Journal: Trends in Food Science & Technology

We are grateful to the Reviewer for several amendments to improve the scientific quality of the manuscript. The Row in black bold refers to the original manuscript. Our answers to the comments/amendments/suggestions raised by the Reviewer are reported after the initials AU:.

Abstract
AU: We modified The abstract as suggested by the Reviewer.

Row 30
AU: This statement was quoted in OECD/FAO 2015. This report describes the market outlook of dairy products up to 2024.

Row 34
AU: We modified the text as follows: "An increase in the production of new varieties including AC, fulfilling the growing demand in developing dairy markets, is expected (PMFood & Dairy Consulting, 2014).". This Ref. reports the growing outlook of cheese market up to 2020.

Rows 37, 38
AU: The statement of lines 36 and 37 was removed. We modified the text accordingly. Revised line 37.

Row 39
AU: We thank the Reviewer and modified the text accordingly. Revised lines 39–40.

Row 44
AU: The text was modified as follows. "Non-dairy AC (largely obtained from vegetable proteins and fats) represent a market opportunity for vegan cheeses.". Revised lines 50–51.

Row 45
AU: The text was modified as follows. "In comparison to natural cheeses, this group of AC is cost saving due to the use of cheaper vegetable oils instead of milk fat and the partial substitution of protein by starch and/or other hydrocolloids. Cost-effectiveness is also attributable to the lower capital cost of manufacturing equipment and the fact that no ripening is required (Guinee, 2011).". Revised lines 43–47.

Row 46

AU: We thank the Reviewer and modified the text accordingly. Revised line 56.

Row 50
AU: The subjext is regulations, thus we did not modify the verb into “classifies”. We modified the other correction. Revised line 60-61.

Row 54
AU: We inserted the Ref.. Revised line 64.

Row 59
AU: We modified the text as suggested. Revised line 69.

Row 60
AU: We modified the text as follows: “Other positive aspects distinguish AC from its natural counterpart, i.e. relatively stable texture, flavor and cooking properties during cold storage, health and dietary benefits, compositional and nutritional flexibility, employment of different functional ingredients, tailored manufacture to customize functionalities, convenient packaging and ease-of-use.”. Revised lines 69–72.

Row 68
AU: We modified the text as suggested. Revised lines 80–81.

Row 70
AU: We modified the text as suggested. Revised lines 82–83.

Row 72
AU: We modified the text as suggested. Revised line 85.

Row 80
AU: This statement was removed in the revised manuscript.

Row 82
AU: This statement was removed in the revised manuscript. Revised line 94.

Row 82
AU: In this context, the term “type” refers to the various ingredients of AC not to intra-variability. For instance, we do not refer to different types of fat, i.e. dairy or vegetable. Again, we do not refer to different types of starch, i.e. native, modified. In the same way do not refer to types of water. To avoid misunderstanding, we removed “water”.

Row 83
AU: We thank the Reviewer for Her/His detailed corrections. We modified the text as suggested. We removed the term water. Revised line 95.

Row 87
AU: We removed Ref. McIntyre et al. (2017a) and the sentence.

Row 89
AU: We thank the Reviewer. Clean of sliceability is the functionality to cut cleanly AC into thin slices without fracturing or crumbling or sticking to cutting implement. Really, we intended to list some functional properties of unheated AC. We modified the sentence. "…sliceability and shreddability …". Revised line 101.

Row 93
AU: We thank the Reviewer for Her/His detailed corrections. We modified the text as follows: "…stretchability (the latter for pasta filata varieties) …". Revised lines 104−105.

Row 94
AU: We thank the Reviewer for Her/His comment. We deleted: softness.

Row 98
AU: We thank the Reviewer. We inserted the term ingredients. Revised lines 110.

Row 100
AU: We introduced 2 Refs. concerning the use of emulsifiers (Jana & Upadhyay, 2001; Jana, Upadhyay, & Solanky, 2005). Revised lines 111−112.

Row 102
AU: We did not insert the suggested specification. Unfortunately, we did not find any Ref. dealing with the addition of little amounts of cheeses in AC formulation. We suppose ripened cheeses are preferred.
We deleted the term "strength".

Row 105
AU: The sentence was removed.

Row 107
AU: The sentence was removed.

Row 112

AU: We modified the text. Revised line 124.

Row 114
AU: We modified the text as follows: "Noronha, O'Riordan, and O'Sullivan (2007) in laboratory-made AC reported moisture levels up to 60%. These AC resulted soft or tough as a function of the amount of resistant starch added.". Revised lines 126−128.

Row 117
AU: We thank the Reviewer for the correction. We deleted the sentence. Really, proteins behave as gelling and emulsifying agents.

Row 119
AU: We deleted the paragraph.

Row 126
AU: We deleted the paragraph.

Row 128
AU: This was a general statement. We specified protein source in relation to end product in subsequent paragraphs. "Caseinates (especially sodium caseinate) are preferred over CN especially in spreadable imitation cheese products (Guinee, 2009)" (revised lines 208−209) or "The use of rennet CN prevails over other protein sources especially in MCA" (revised line 194).

Row 130
AU: We corrected as suggested.

Row 133
AU: We are grateful to the Reviewer for the amendment. We modified the text as follows: "During mixing of ingredients, water helps in dissolving the ES, resulting in the better chelation of calcium …". Revised line 135−136.

Row 137
AU: We inserted Ref. (Fox et al., 2017b). Revised line 139.

Row 139
AU: We corrected as suggested. Revised line 141.

Rows 140, 141
AU: In this paragraph the authors (Ennis and Mulvihill, 1999) are describing the action mechanism of fat emulsification by protein. The degree of emulsification of the oil phase in

cheese analogues is influenced by the nature and concentration of the calcium-sequestering salt used (Cavalier-Salou & Cheftel, 1991). Increasing the amount of protein capable of interacting with the oil phase by increasing the extent of protein hydration allows the formation of a greater oil phase surface area, than when hydration of the protein is limited. Extensive hydration of the protein molecules permits greater interaction of the protein molecules with the oil droplet surface, giving better stabilisation of the dispersed oil phase than is obtained at low levels of protein hydration.

Row 149
AU: We preferred to delete this paragraph in the revised manuscript.

Rows 144-159
AU: These paragraphs were deleted in the revised manuscript. The research of Hennelly et al. (2006) was discussed in Inulin section. The work of Noronha et al. (2007) was discussed in Starch section.

Row 163
AU: We reported the most recent paper quoting the typical addition level of ingredients in the formulation of AC.

Row 165
AU: This is the first general statement relating the feasibility to substitute starch for protein. In these Refs. the authors evaluated the effect of different starches as protein replacer. In subsequent lines and also under Starch section we provided the reader with details on type of starch adopted.

Rows 166−176
AU: We introduced a detailed paragraph describing the results of the investigation. Revised lines 162−169.

Row 174
AU: We corrected as suggested. Revised line 176.

Row 175
AU: We deleted "nutritional properties" and "rather than for a pleasant or dairy-like flavor".

Row 175
AU: We inserted "CN" as suggested. Revised line 177.

Row 180

AU: We inserted “find” because caseins is the subject. Revised line 232.

Row 185

AU: We inserted “… at rates of ~18−24% …”. Revised line 195. We introduced in the revised manuscript a paragraph on this topic. “Jana, Shah, Aparnathi, and Padhiyar (2015) evaluated the functionality and sensory quality of MCA samples for Pizza topping obtained using amounts of 22.0%, 24.5% or 27.0% of rennet CN in the formulation. The MCA sample with 24.5% rennet CN was preferred for the meltability (Schrieber test), the moderate fat leakage and the adequate stretchability. Sensory analysis carried out through hedonic tests measured appearance, flavor, melting and chewiness attributes of AC. The panel of assessors significantly preferred the MCA sample with 22.0% rennet CN, whereas the sample obtained with 27.0% CN received the least scores.” Revised lines 198−205.

Rows 188, 189

AU: We introduced this paragraph: “Anyway, acid CN is reported to hydrate better than rennet CN when converted to sodium or calcium caseinate by alkalinization because its water binding capacity is enhanced (Guinee, 2011). Some authors (Gustaw & Mleko, 2007; Sołowiej et al., 2015) reported the suitability of acid CN as the major protein source (10−13% w/w in the formulation) in the manufacture of processed cheese analogues (See also sections Hydrocolloids and Preservatives).”. Revised lines 189−193.

Row 190

AU: We specified the functionality, i.e. the water binding capacity. We modified the text as reported in the previous comment.

Row 191

AU: In the previous paragraph we reported the typical “industrial” use of acid CN reported by Guinee (2011). Gustaw & Mleko (2007) and Sołowiej et al. (2015) reported under their specific conditions the feasibility to use acid CN at higher concentrations in the formulation. We described with a great deal of details these investigations in sections Hydrocolloids and Preservatives).

Rows 191, 198

AU: These rows were deleted in the revised manuscript.

Rows 193, 194

AU: We deleted the work of Hoffmann, Hinrichs, Johannsen, Scheurer, and Maurer-Rothmann (2005) in the revised manuscript.

Row 197

AU: We thank the Reviewer for Her/His specification. The Reviewer refers to authors using acid-CN as protein source. They did not report any problem of acid-CN solubilization when preparing AC (neither directly nor an extension of processing time). We suppose that, under the conditions they adopted, the use of disodium phosphate (0.8%) increasing the pH of the dispersion exerted a positive role in favoring the solubilization of acid-CN.

Row 201

AU: We thank the Reviewer and we deleted the functional properties of MCA.

Row 190

AU: Also bland flavor and pale color refers to AC. Really the meaning of our sentence was the following: "Rennet casein is the primary protein source used in the manufacture of mozzarella cheese analogues, mainly because the latter result with a bland flavor, pale color and superior functional properties (shredding, stretching and melting) when compared to analogues made with proteins from other sources.". Anyway as suggested we removed the sentence reported in the original manuscript.

Row 204

AU: We thank the Reviewer and we deleted the term stringiness. We did not insert the subsequent modification of the Referee, because in the revised manuscript the final part of the sentence was removed. Really, the comparison of MC and MCA properties was described in detail in the new section "Functional properties of analogue cheeses *vs* natural cheeses and changes upon storage"

Row 209

AU: We introduced the abbreviation in a previous row in the revised line 195.

Row 209

AU: O'Riordan et al. (2011) reported in the book chapter of Tamime that AC produced only on the base of Na caseinate are undesirable as a pizza topping because of poor meltability and firmness.

Rows 214-218

AU: We modified the paragraph. We reported only some results of this investigation. In particular, we chose the most relevant and clear outcomes. Also the authors reported that some evidences of their work were not clear. The cheese is a spreadable variety. We modified the text as follows: "The use of other dairy derivatives or the combination of different protein sources were useful strategies to pursue specific functionalities and received a deal of attention in past years. For instance, the partial substitution of sodium caseinate (90% protein) with the same amount of whey protein concentrate (WPC, 35% protein) (at rate of 1.5%) caused slight increase in AC hardness and a reduction of both meltability and sensory properties (measured by hedonic ratings of untrained panellists) in comparison to sodium caseinate-based AC (Hosseini, Habibi Najafi, & Mohebbi, 2014). The trial AC sample was slightly lower in protein content in comparison to the control (21.4% vs 22.2%). The negative effects on sensory properties were offset by addition of 0.3% guar gum in the formulation.”. Revised line 210−218.

Rows 219, 220

AU: We preferred to remove the work quoted in the review of Bachmann (2001).
Actually, this is a study of Abou El Nour, Scheurer, and Buchheim (1996) who investigated on the use of a casein-whey protein co-precipitate. The authors partially replaced (up to 50%) rennet CN with above protein derivative in AC formulation to obtain a block-type processed cheese analogue. As a consequence, cheese firmness increased and flowability of the melted product decreased to an extent that increased with the level of substitution. This negative functionality (i.e. the adverse effect on meltability) was partly associated to the uncontrolled heat-induced denaturation and aggregation of whey proteins and the subsequent formation of large aggregates of whey protein-casein, which affected the size/gelation capacity of the co-precipitate.

Rows 221, 222

AU: We reported in detail the results of these authors. The text was modified as follows: “Dhanraj et al. (2017) studied the influence of WPC as partial rennet CN replacer in the formulation of MCA used as pizza topping. Blends of rennet CN:WPC (68% protein) at ratios 95:15, 90:10, 95:5 were evaluated in terms of baking and sensory properties. MCA samples of different protein blends showed only slight differences in terms of shredding, meltability (Schrieber test), stretch test (Fork test) and total sensory scores (hedonic tests evaluating appearance, flavor, melting, and chewiness attributes). The sample with protein blend at CN:WPC ratio 90:10 was slightly preferred over the other blends.”. Revised lines 226−232.

Rows 224, 225

AU: We preferred to remove this Ref.. We introduced more details on a recent paper (Dhanraj et al. (2017) on the use of WPC as rennet CN replacer. Revised lines 226−232.

Rows 226

AU: We modified the text as follows: “Whey proteins (WP) derivatives (typically WPC), as the alternative to CN for nutritional and economic reasons, exert complex effects on the properties of AC.”. Revised lines 219–220.

Rows 228

AU: We thank the Reviewer and modified the text as suggested.

Rows 228, 229, 230

AU: The following sentence "Generally, inclusion of WP results in a gel network more deformable, holding more water, with increased firmness and reduced meltability (Guinee, Carić, & Kaláb, 2004; Mleko & Foegeding, 2000; Mleko & Foegeding, 2001)." was removed.

Rows 232, 233

AU: This sentence is a general statement quoted by Guinee et al. (2011). It allows the readers to understand the action mechanism of the addition of WP derivatives (typically WPC) in rennet-CN based AC. Following the suggestion of the Reviewer, we modified the text as follows: "A limited addition of whey-based derivatives (typically WPC), restricted from 1% to 3% of the formulation of rennet CN-based AC, was suggested in those applications where flow-resistance is envisaged (e.g. burgers) (Guinee, 2011)". Revised lines 223−225. Results of additional investigations (Dhanraj et al., 2017 or Lee, Huss, Klostermeyer, & Anema, 2013) on this topic were subsequently detailed in the revised manuscript.

Row 236

AU: We corrected and rephrased the results obtained by Sołowiej et al. (2014). The revised text is as follows: "Sołowiej et al. (2014) focused on the influence of increasing amounts (from 1% to 3%) of high-protein derived products (i.e., WPI at 94% protein or WPC at 80% protein) as protein replacers in a processed cheese analogue obtained from rennet CN (at protein rates ranging from 10% to 13%). In comparison to samples obtained using rennet CN alone, AC with added WP derivatives showed progressive increase in hardness, adhesiveness and viscosity, while meltability decreased. Analogue cheeses with WP derivatives were good for shredding, but with poorer melting properties. These results pointed out that the use of the appropriate combination of CN and WP is a tool to control textural/rheological properties and meltability of AC.". Revised lines 232–239.

Rows 240-242

AU: We rephrased the sentence referring only to the investigation of Lee, Huss, Klostermeyer, & Anema (2013). The revised text is as follows: "The addition of WPC (80% protein) solutions at different degrees of denaturation (by heating at temperatures from 67 °C to 85 °C for holding times of 150 s in a scraped-surface exchanger) in the formulation affected the rheology and microstructure of rennet CN-based spreadable AC (Lee, Huss, Klostermeyer, & Anema, 2013). Degrees of denaturation in AC with microparticulated aggregates ranged from 40% to 100%. Higher levels of WP denaturation in AC caused a decrease in hardness and an increase in the meltability of the resultant spreadable AC compared to samples with unheated WPC solution. This effect was attributed to the incorporation of denatured WP as relatively large particles that did not contribute to the matrix structure, thus disrupting the AC network. In contrast, following the addition of native WP, the manufacturing conditions of AC promoted a only partial denaturation (~ 40%) of WP resulting in their subsequent incorporation into the matrix, producing a stronger structure.”. Revised lines 240–249.

Rows 245, 246, 247

AU: We thank the Reviewer for Her/His amendments. Anyway, we preferred to remove this Ref. (Sołowiej, Mleko & Gustaw, 2008).

Rows 248-253

AU: We thank again the Reviewer for Her/His amendments. We preferred to remove also this Ref. (Sołowiej, Mleko, Gustaw, & Udeh, 2010).

Rows 254-258

AU: We thank the Reviewer for the comments. We preferred to remove the Ref.. The protein of this AC consisted only of WP from whey of hard or semi-hard cheeses.

Rows 259

AU: We specified the limitations as follows: “Due to the high cost and some functionality limitations of CN (e.g. limited hydration capacity and solubility in water), …”. Revised lines 250–251.

Row 261

AU: We corrected as suggested by the Reviewer.

Row 265

AU: We corrected as suggested by the Reviewer.

Row 268, 269

AU: We thank the Reviewer for the comments. We introduced a new short paragraph. The revised text is as follows: “At room temperature, about 15% of the milkfat should still be a solid forming an exterior crystalline layer, while the oil in the interior of the fat globules. The solid component consists of a network of triglyceride crystals connected by Van der Waals forces of attraction. Following ES addition, shearing and heating actions during AC manufacture, the rupture of these bonds takes place, forming melted oil droplets of variable diameter, which are emulsified by the protein (Lobato-Calleros, Vernon-Carter, & Hornelas-Uribe, 1998).”. Revised lines 261–266.

Row 269

AU: We corrected as suggested.

Row 271

AU: We thank the Reviewer for improving the writing style. We modified the term.

Row 273
AU: We corrected as suggested.

Row 274
AU: We deleted butteroil.

Row 274
AU: We modified the sentence as suggested. "Conversely, in AC categorized as partial dairy, the oil/fat source may be milk fat in addition to vegetable oil/fat.". Revised lines 271–272.

Row 275
AU: We specified as suggested. Revised line 274.

Row 287
AU: We modified the text as suggested by the Reviewer. The revised text is as follows: "Generally, the total or partial replacement of milk fat by vegetable oils (up to 50%) (liquid at room temperature) resulted in softer AC (Budiman, et al., 2000; Cunha, Dias, & Viotto, 2010; Shabani, Sarfarazi, Mirzaei, & Jafari, 2016)". Revised lines 280–283.

Row 289
AU: We thank the Reviewer and corrected as suggested. Revised lines 283–284. We mentioned melting points of vegetable fat (revised lines 286–290).

Row 291
AU: We thank the Reviewer and corrected as suggested. Revised line 297.

Row 294
AU: We thank the Reviewer and corrected as suggested. Revised line 299.

Row 298
AU: We thank the Reviewer. Anyway, this statement was removed in the revised manuscript. We detailed the type of fiber used in the formulation in another section (Inulin).

Row 303
AU: The abbreviation was already introduced previously. Revised line 39.

Row 307

AU: We thank the Reviewer and corrected into: "Types and properties of different ES have been reviewed (Lucey, Maurer-Rothmann, & Kaliappan, 2011).". Revised lines 311−312.

Row 307
AU: In Table 1 we reported the typical levels of ES in the formulation of different types of AC. We modified the sentence as follows: "Various ES are used …". Revised line 313.

Row 309
AU: We thank the Reviewer and corrected the text. Revised line 314.

Row 310
AU: We thank the Reviewer. The rate of addition was reported in revised Table 1. We modified the text as follows: "Typically, a blend of phosphates (disodium phosphate) and citrates (trisodium citrate) in the ratio of 1:1 is used in AC manufacture (El-Bakry et al., 2011b). Typical levels of ES used in formulation of AC are reported in Table 1.". Revised lines 314–316.

Row 312
AU: We thank the Reviewer but this sentence was removed in the revised text.

Row 314
AU: We thank the Reviewer and corrected as suggested. Revised line 317−318.

Row 318
AU: We thank the Reviewer for improving the writing style. We corrected as suggested. Revised line 321.

Row 320
AU: We removed the sentence as suggested.

Row 321
AU: We rephrased the sentence introducing more details on this investigation. The revised text is as follows: "McIntyre, O'Sullivan, and O'Riordan (2016) evaluated the effect of different types (disodium phosphate and trisodium citrate), concentrations (10 and 30 mmol L^{-1}) and ratios (1:0, 2:1, 1:1, 1:2 and 0:1) of ES in rennet CN dispersions (15%), …". Revised lines 335–337.

Row 328
AU: We corrected as suggested. Revised line 348.

Row 331
AU: We corrected as suggested. Revised line 352.

Rows 335, 336

AU: We thank the Reviewer for Her/His corrections. We corrected as follows: “El-Bakry, Duggan, O'Riordan, and O'Sullivan (2010) investigated the impact of decreasing sodium in AC, through reduction of ES concentration in the formulation from a standard level of 1.4% up to about 0.8%. In the standard AC the ES mixture consisted of trisodium citrate and disodium phosphate at a ratio of 2.16:1. Over the range of ES reductions, a progressive increase in processing time and in the mixing energy needed to emulsify the fat phase. As a consequence, a decrease of fat globule diameter was observed. The prolonged mixing during manufacture increased hardness and decreased the heat-induced flowability. Only a ES reduction of up to 20% was judged feasible, being above AC functionalities only slightly altered and processing times increased by only 25 %.” Revised lines 353−360.

Row 337

AU: We corrected the text as suggested.

Row 339

AU: We thank the Reviewer for Her/His corrections. We modified the text focusing on the obtained by El-Bakry et al. and modified the text as follows: “A reduction of sodium content was achievable also by the replacement of sodium ES blend (disodium phosphate and trisodium citrate, at ratio 0.46:1.0) with their potassium equivalents (El-Bakry et al., 2011a) in a 48% moisture rennet-based AC. This substitution led to better protein hydration which required less mixing energy of AC ingredients. Adding potassium-ES (instead of sodium-ES) resulted in a slight increase in pH value (from 6.0 to 6.3) due to less potassium binding (with respect to sodium) to CN and to a less displacement effect of hydrogen ions of CN. The increased pH was hypothesized to increase electrostatic repulsion between the CN molecules, thus improving protein hydration and, as a consequence better fat emulsification. Differences were observed during refrigerated storage in functional properties. The increases in pH value and fat globule size promoted slight decrease in hardness, a less cohesive structure and slight increase in flowability and adhesiveness. The increase in pH value of AC obtained with potassium ES was hypothesized as the cause of their reduced microbial stability during 12 weeks storage (at 4 °C) in comparison to the sodium equivalents. The trial was carried out at laboratory-scale and thus under hygienic conditions not as strict as in industrial processing (i.e. under controlled air flow and using aseptic packaging). After manufacture, the sodium AC (pH 6.0) showed a total viable count of ~ 10^1 ufc g^{-1}, which slightly increases up to 10^2 cfu g^{-1} throughout the storage period. The substitution of the sodium salts by their potassium equivalents led to substantial higher counts (up to10^4 cfu g^{-1}). In either formulations no mould or yeast growth was detected.”. Revised lines 360−377.

Row 343-346

AU: We thank the Reviewer for Her/His corrections, but the paragraph dealing with the work of Schatz et al. was removed in the revised text.

Row 349

AU: We thank the Reviewer for Her/His corrections. We modified the text as follows: "".Native starch is added to AC formulations at levels of 2–4% to substitute ~ 10–15% rennet CN for its relatively low cost, availability and benefits (Considine et al., 2011; Guinee, 2011). Revised lines 443–444.

Row 352

AU: We thank the Reviewer and introduced a detailed paragraph on the subject. The revised text is as follows: "The partial replacement (15%) of rennet CN with wheat, maize, waxy-maize or rice starches resulted in AC with fat globule diameters (2–10 μm) smaller than the starch-free control, thus indicating more extensive fat emulsification (Mounsey & O'Riordan, 2001). The authors observed that during AC manufacture the oil of starch-containing AC was emulsified more rapidly than the control. The starch likely behaved as a filler in the early, low-temperature stages of AC manufacture, leading to increased availability of water to hydrate the CN, and subsequently improved fat emulsification. An important impact of partial substitution of rennet CN with starch was the reduction in AC meltability in the order maize > waxy-maize > wheat > rice (Mounsey & O'Riordan, 2001, 2008d). This effect was attributed to different factors such as fat globule size and the disruption of protein matrix by swollen starch granules. The latter favoring the immobilization of water by the granules may have resulted in the dehydration of the protein matrix, which led to increased hydrophobic protein-protein interactions and ultimately poorer meltability. The protein dehydration was likely responsible of a honeycomb structure of the protein matrix in the area immediately adjacent to the starch structure. Rice starch with its small granule diameter, relatively low amylose content (13% *vs* 25% and 28% of maize and wheat, respectively) and small swelling capacity had the least effect on melting reduction, resulting in an AC with acceptable rheological properties.". Revised lines 395–410.

Row 359

AU: We thank the Reviewer and modified the text. Revised lines 411–412.

Row 362

AU: We thank the Reviewer, but we deleted this sentence in the revised text.

Row 363

AU: We thank the Reviewer. We specified amylose content as follows: "Starches with high amylose content (25% maize, 28% wheat) resulted …". Revised line 412.

Row 365

AU: We thank the Reviewer. We introduced a detailed paragraph. The revised text is as follows: "Pre-gelatinized starches (of maize, waxy-maize, wheat, potato or rice) were used at rates of 3% as a replacer of 15% rennet CN in the formulation of AC (with 17% protein *vs* 20% protein of the starch-free control) (Mounsey & O'Riordan, 2008b). Analogue cheeses with above pre-gelatinized starches showed poorer fat emulsification resulting in softer, less cohesive AC that had reduced meltability, in comparison to the control AC. The inclusion of pre-gelatinized starch in AC would only be useful when low meltability in a heated system is required (deep fried cheese) or in a ‘‘cold" application (salad bar) (Mounsey & O'Riordan, 2008c)." Revised lines 415–422.

Row 370, 371

AU: We thank the Reviewer, but we preferred to report Trivedi et al. (2008) only in revised Table 2.

Row 376, 377

AU: We modified the text introducing a more detailed paragraph. The revised text is as follows: "Resistant starch was investigated as a source of dietary fiber to provide AC with the image of a health promoting and more nutritious food. Noronha et al. (2007) in an AC with 52% moisture and 12% fat contents replaced about 50% fat, consisting of hydrogenated palm oil and rapeseed oil (in the ratio 2:1), with native resistant starch. The observed excessive hardening effect was reduced by increasing the moisture of AC to 60%. The authors verified that at this moisture level the further replacement of up to 90% of fat with starch allowed to obtain a 2% fat AC with hardness and cohesion values amenable to slicing, shredding and moulding. This research demonstrated that a high moisture content (60%) plasticized AC matrix allowing the cheese to melt even if it contained only 2% fat. After sensory analysis by ranking tests, this high-moisture low-fat AC lost its grainy texture and panellists perceived it as smoother and firmer in comparison to samples where up to 85% fat content was replaced. The improved mouthfeel was attributed to the fact that more abundant and smaller starch particles in the 2% fat cheese may not have absorbed as much water as particles in the cheeses containing lower levels of starch.". Revised lines 423–434.

Row 377, 378

AU: We thank the Reviewer. We introduced more details on this investigation and moved it in the section "Miscellaneous food additives". The text in the revised manuscript is as follows:
"Noronha, Cronin, O'Riordan, & O'Sullivan (2008a, 2008b) reported that the quality of EMC-flavored AC is affected by various parameters, such as the amount of flavor-active hydrolysis substances in the EMC (i.e. free fatty acids), the pH and the composition of AC. In details, these authors analyzed medium (13%) and low (2%) fat AC (pH 6.0 or 5.5) flavoured with 5% EMC at 16%, 28% or 47% total free fatty acids (low to high levels of hydrolysis, respectively). Following sensory analysis by ranking tests, the acceptability by panellists of low (2%) fat AC

was optimized by adding EMC with medium level (28% total free fatty acids) of lipolysis. ‘Stronger’ flavors were perceived in AC with pH of 5.5, because of the higher levels of free fatty acids than those formulated with a higher pH. Irrespective of the degree of hydrolysis of the EMC used, AC with 13% fat showed higher flavor release properties than those containing 2% fat. Low-fat cheeses flavored (pH 6.0 or 5.5) with 28% lipolysis EMC were described as ‘well-balanced’ and ‘cheesy’ and were preferred over AC containing high hydrolysis EMC.”. Revised lines 554–564.

Row 390

AU: We thank the Reviewer for the correction. We explained the unclear sentence in a new paragraph. The revised text is as follows: “The resistant starch used, i.e. high amylose corn starch has a low water-holding capacity and does not swell during microwaving due to the insufficient water in the vicinity of the granules as most of it evaporates quickly due to the rapid nature of microwave heating. Moisture exerts a primary role also during pre-expansion cold storage time. The authors observed (by nuclear magnetic resonance relaxometry) that tightly bound water decreased during AC cold storage (up to 9 days). Such phenomenon was attributed to an increase in water mobility. The more mobile water increased the plasticization of the AC matrix making it more stretchable under pressure during microwaving. After 9 days storage the volumetric expansion of 60% moisture AC increased about 4-fold in comparison to 45% moisture AC.”. Revised lines 443–451.

Row 404

AU: We thank the Reviewer for the suggestion. We modified the text with new detailed results relative to the research of Hennelly et al. (2006). The revised text is as follows: “The simplest method of incorporation in AC would be as dry powder, but in this way its inclusion would lead to hard lumps and free water. To improve the distribution of inulin in AC, Hennelly et al. (2006) studied the effect of incorporating inulin either as a heated solution (80 °C) or as a shear-induced gel to replace 63% hydrogenated palm oil in a 54% moisture AC. The fat content was 11.0% in AC samples with 3.4% inulin, while in the control sample (inulin-free) was 21.7%. As a result of fat replacement, rennet CN content passed from 20.1% in the control sample to 23.8% in AC containing inulin. The inulin incorporation as a gel or as a solution had no significant effect in terms of AC microstructure and meltability in comparison to the control sample. The presence of inulin in AC caused a significant increase in hardness in comparison to inulin-free samples. Such result was apparently unexpected because hydrogenated palm oil is a hard fat (melting point 48 °C). Its substitution with a soft inulin gel or solution would be expected to result in a decrease in hardness. Being unchanged AC moisture level (54%), this virtual softening was more than offset by the increase of protein:fat and protein:moisture ratios of inulin samples with respect to control sample.”. Revised lines 457–469.

Row 415

AU: We thank the Reviewer for Her/His detailed correction. We deleted "emulsifying". Anyway, basil seed gum was reported to possess emulsifying properties. In a subsequent paragraph we reported: "Hosseini-Parvar et al. (2015) explored the impact of basil seed gum (a surface-active polysaccharide-based hydrocolloid) on functional properties of AC. Analogue cheeses (52% moisture, 30% soy oil contents) were manufactured at different levels of proteins (6–10%), water (56–52%) and basil seed gum (0–1%). Increasing levels of added hydrocolloid in the formulations with the same protein content contributed to oil emulsification. By adding basil seed gum, it was possible to manufacture AC with higher firmness, but slightly lower meltability, and at lower cost owing to the lower protein and higher moisture contents. For instance, replacing 4% of the protein content (i.e. from 10% to 6%) with 0.1% basil seed gum and 4% water (from 52% to 56%) allowed to obtain an AC with slightly lower meltability than the sample with 10% protein (0% basil seed gum and 52% moisture contents). The rationale was the formation of a hydrocolloid network capable to strengthen the network formed by casein strands..". Revised lines 499−509.

Row 425, 427

AU: We thank again the Reviewer and introduced more details on this investigation. The revised text is as follows: "Acid CN-based processed cheese analogues (11.5% acid CN in the formulation) obtained with addition of WPI (0.5% in the formulation) and different hydrocolloids blends (up to 0.21% in the formulation) showed increased hardness and decreased adhesiveness (Gustaw & Mleko, 2007). The magnitude of these modifications varied as a function of the amount of hydrocolloids blends. The addition of κ-carrageenan:locust bean gum (ratio 1:1) or xanthan gum:locust bean gum (ratio 1:1) blends in the range 0.07–0.21 caused a linear increase in AC hardness and a decrease in adhesiveness independently of the blend used. These effects were explained by the synergistic interaction between these polysaccharides. Probably the interaction between xanthan gum or carrageenan with galactomannan was stronger than the polysaccharide with CN and ultimately polysaccharides created a separate gel network.". Revised lines 489−498.

Row 429

AU: We are grateful to the Reviewer for Her/His amendment, bur this Ref. was deleted in the revised text.

Row 435

AU: We deleted this Ref.. Liu, Xu, and Guo (2008) used low-methoxylated pectin. "Pectin gel was prepared by mixing the pectin with water and interacted with calcium ion to form a weak-gel". The author provided no conditions on pectin gel preparation.

Row 443, 444

AU: We are grateful to the Reviewer for Her/His amendment. We corrected the text.

“The use of little amounts of cheese (<50 mg/kg of final product, Fox et al., 2017a) is a way to impart flavor. An alternative is the use of dairy cream instead of vegetable oil/fat.”. Revised lines 544−546.

Row 447

AU: We are grateful to the Reviewer and modified the text. “Flavor compounds include enzyme-modified cheeses (EMC), smoke extracts and starter distillates, whose typical addition level ranges from 0.5% to 3.0% (Guinee, 2007).”. Revised lines 547−549.

Row 451

AU: We changed the text. “Composition and flavor components of EMC have been reviewed by Kilcawley, Wilkinson, & Fox (1998).”. Revised lines 627−628.

Row 453

AU: We removed results obtained by El-Bakry et al. (2011d) on sensory analyses, as suggested. The investigation of Gustaw and Mleko (2007) on the effect of NaCl on texture properties was detailed to improve comprehension. Revised lines 568−574.

Row 464

AU: We reported pH interval as suggested. “AC stability would rapidly decrease at pH levels of the homogenous hot molten blend (~ 8–9) (Guinee, 2007).”. Revised lines 512−513. The term blend in this context refers to the hot mass of ingredients. We are explaining the aim and the importance of reducing the pH level of the hot molten mass before cooling.

Rows 466, 467

AU: We modified the text as follows: “Thus, the addition of acidifying agents allows the lowering of pH value, to average values of 6.0–6.6 for a generic AC (Fig. 2). Guinee (2011) reported that, as a function of the final end-use application, the final pH value may significantly vary within the same variety. For instance, in the case of MCA the final pH may range from 6.1 to 6.6.”. Revised lines 514−517.

We added also: “The acids most frequently added are citric or lactic acid. Food-grade organic acids are used at concentrations of ~ 0.2–1%, as a function of the type of acid (see also Table 1). Also types of ES, having different pH-buffering capacity, affect subsequently the concentration of acidulants added in the formulation.”. Revised lines 519−522.

Row 469

AU: We removed the sentence.

Row 472

AU: This sentence was removed in the revised manuscript.

Row 473

AU: We discussed the role of NaCl in sections "Preservatives" and "Miscellaneous and food additives".

Row 473

AU: On average the level of preservatives in AC formulation is 0.1% (Guinee, 2007). In addition we reported some indication on the maximum levels permitted in AC, according to FAO/WHO Codex Stan 1992–1995 (2016). Under the section "Miscellaneous food additives" in the revised version of the manuscript we reported the following sentence: "A steadily revised list of permitted food additives including the maximum use levels in AC was provided by the Commission of Codex Alimentarius (2016). The functional classes reported in the list include acidity regulators, colors, emulsifying agents, flavor enhancers, preservatives and stabilizers.". Revised lines 595–598.

Row 478

AU: We thank the Reviewer and following Her/His suggestion we introduced a detailed description of this investigation. "Recently, Khanipour et al. (2016), testing different combinations of preservatives (NaCl, potassium sorbate, nisin), developed a logistic regression model enabling prediction of the probability of growth of *Clostridium sporogenes* in a high moisture (60%) ambient shelf-stable processed cheese analogue. This microorganism was selected having similar growth characteristics to the pathogen *C. botulinum*, but not being dangerous. They outlined that NaCl at 3% (a relatively high concentration) alone did not prevent the growth of *C. sporogenes* in the high moisture AC samples (at pH 5.5). Differently, proper combinations of NaCl (up to 3%), potassium sorbate (up to 0.2 %) and nisin (up to 240 ppm) were successful inhibitors. The model enabled the level of probability to be set to provide a required level of stringency.". Revised lines 530–538.

Row 480

AU: We removed sweeteners. Really, sweeteners are hardly used in AC manufacture. Anyway, in FAO/WHO Codex Stan 1992–1995 (2016) the maximum amounts permitted for this class of additives ranges from 33 ppm for neotame up to 1,000 ppm for aspartame.

Row 483

AU: We specified the mineral preparations used in AC. "Mineral and vitamin preparations introduced as nutritional supplements (at mean rates of 0–0.5%) (Chandan & Kapoor, 2011), include magnesium oxide, zinc oxide, iron, vitamin A, palmitate, riboflavin, thiamine and folic acid (Guinee, 2011).". Revised lines 600−603.

Row 484

AU: To our knowledge, there are no international scientific papers reporting the level of vitamins specifically in AC. We suppose this aspect may be reported in patents on Ac manufacture, being a relevant information for manufacturers.

Row 493
AU: We modified the text as suggested. Revised line 608.

Row 494
AU: We agree with the Reviewer. We are aware that there are several scientific works reporting the flow-sheet of specific AC varieties. In addition, there are also several patents on the subject. Anyway, we referred to Fox et al. (2017b) because these authors provided the reader with a general description of the manufacture protocol.

Row 499, 502
AU: We modified the text as follows: "Schatz et al. (2014) recommended a procedure to pre-disperse the CN and hydrate dry ingredients in hot solution, followed by addition of ES. This procedure allows obtaining AC with proper consistency, good flow of the melt and no impairment of melting properties.". Revised lines 614−617.

Row 505
AU: Really, we modified as follows: "The latter, reducing the free interfacial energy of the fat phase, emulsifies the dispersed oil droplets during processing, thus contributing to the formation of a stable oil-in-water emulsion.". Revised lines 620−622.

Row 506
AU: We modified the text.

Row 508
AU: We modified the text. "Cooking conditions (time and temperatures) have an influence on the functional properties of the final product (Shabani et al., 2016).". Revised lines 626−627.

Row 510
AU: We changed the text. "… AC spreads …". Revised line 629.

Row 511
AU: We changed the text as suggested.

Row 518
AU: We modified as suggested. Revised lines 638−639.

Row 521
AU: We deleted this sentence in the revised version.

Row 523
AU: We thank the Reviewer and specified speeds in the text. Revised lines 639−645.

Row 527
AU: We reported a range 0.1-1.0%. Please, see also revised lines 571−574. "The acids most frequently added are citric or lactic acid. Food-grade organic acids are used at concentrations of ~ 0.2–1%, as a function of the type of acid (see also Table 1). Also types of ES, having different pH-buffering capacity, affect subsequently the concentration of acidulants added in the formulation.". Revised lines 519−522.

Row 530
AU: We thank the Reviewer and modified the text. Revised line 651.

Row 531
AU: Upon cooling the viscous mass is transformed either in a soft and spreadable body or in a firm and sliceable body. We think that the sentence we reported in the original manuscript is correct. We separated cooling step from storage conditions, as suggested.

Discussion are missing on the following very important aspects

1. **Functional properties expected from the AC based on the cheese it is trying to mimic (e.g. stretch for Mozzarella/pizza cheese analogue)**
2. **Textural properties of AC vis a vis natural cheese**
3. **Microbiology of AC vis a vis natural cheese**
4. **Shelf life of AC and changes in functional properties of AC during its refrigerated storage**
5. **Cost aspects**

AU: We are grateful to the Reviewer for Her/His detailed suggestions to improve the quality of the manuscript. For the sake of conciseness (there is an editorial limit of 10,000 words for reviews), we inserted a new Section ("Functional properties of analogue cheeses *vs* natural cheeses and changes upon storage", revised lines 657–711) dealing with topics 1 to 4.
Additional aspects on microbial stability were reported in revised lines 370–377 and 587−591.
Cost aspects were inserted in revised lines 43–50, as follows: "In comparison to natural cheeses, this group of AC is cost saving due to the use of cheaper vegetable oils instead of milk fat and the partial substitution of protein by starch and/or other hydrocolloids. Cost-effectiveness is also attributable to the lower capital cost of manufacturing equipment and the fact that no ripening is required (Guinee, 2011). AC are amenable to incorporate valued functional ingredients. They show relatively stable textural, flavor and cooking properties during cold storage. Additional

advantages of AC lies in compositional and nutritional flexibility, health and dietary benefits, tailored manufacture to customize functionalities (textural, cooking) and finally convenient packing and ease-of-use.

Not even one formulation of Analogue Cheese has been given in the entire manuscript. Authors can give Table containing formulations standardized by few research workers.
AU: We introduced a new table (revised Table 1) including examples of formulations for specific AC types.

Row 538
AU: We inserted the word, as suggested. Revised line 718.

Row 541
AU: We modified the text, as suggested. Revised line 719.

Row 585
AU: In the revised version this Ref. was removed.

Row 586
AU: We deleted "International", as suggested. Revised line 757.

Row 586
AU: We modified the text in Italics, as suggested. Revised line 766.

Row 606
AU: We modified the order of Refs., as suggested. Revised lines 776−781.

Rows 618-621
AU: We modified the text, as suggested. Revised lines 796−799.

Row 625
AU: We inserted page numbers, as suggested. Revised line 805.

Row 627
AU: We corrected the text, as suggested. Revised line 807.

Row 629
AU: We corrected the text, as suggested. Revised line 810.

Row 663

AU: In the revised version this Ref. was removed.

Row 670
AU: We modified the text in Italics, as suggested. Revised line 848.

Row 706
AU: In the revised version this Ref. was removed.

Row 729
AU: We moved Noronha et al. (2007) as first Ref., as suggested. Revised line 890.

Rows 730−745
AU: We modified the sequence of Refs, as suggested. Revised lines 892−902.

Row 735
AU: In the revised version this Ref. was removed.

Row 746
AU: We inserted the title of book, pages and chapter number. Revised line 904.

Row 763
AU: We corrected the text, as suggested. Revised line 928.

Row 765
AU: In the revised version this Ref. was removed.

Rows 766-781
AU: We modified the sequence of Refs., as suggested. Revised lines 930−940.

Row 785
AU: This is a book, not a paper.

Row 799
AU: We corrected the title of figure 2, as suggested.

Table 1
AU: We included Table 1 in the text, as follows: "In comparison to natural cheeses, this group of AC is cost saving due to the use of cheaper vegetable oils instead of milk fat and the partial substitution of protein by starch and/or other hydrocolloids. Cost-effectiveness is also attributable to the lower capital cost of manufacturing equipment and the fact that no ripening is

required (Guinee, 2011). AC are amenable to incorporate valued functional ingredients. They show relatively stable textural, flavor and cooking properties during cold storage. Additional advantages of AC lies in compositional and nutritional flexibility, health and dietary benefits, tailored manufacture to customize functionalities (textural, cooking) and finally convenient packing and ease-of-use.”. Revised lines 43−50.

Table 2

AU: We are really grateful to the Reviewer for all corrections introduced in Table 2.
Anyway, in the text of the revised manuscript we introduced several additional details on some investigations. Thus, we preferred to modify the format of the table, removing the column dealing with results. We introduced a revised Table 2 entitled “Recent research topics in analogue cheese formulations and manufacture”. In column 1 we reported the most recent topics of interest and in column 2 we quoted the corresponding Refs.

Figure 1

AU: We deleted the terms softness and flow resistance, as suggested.

Figure 2

AU: We thank the Reviewer for the suggested improvements. To keep the style of figure, we preferred to introduce all suggested modifications in the revised text.
Please, see revised lines 612−613 for dry ingredients,
revised lines 622−623 for type of oils,
revised lines 285, 287, 290, 466 for melting points and
revised lines 514−517 for pH values.

HIGHLIGHTS

- Analogue cheeses are cheese-like products used for consumption or as ingredients.
- A wide array of ingredients are exploitable in analogue cheese manufacture.
- Analogue cheese customization to specific end-product functionalities.
- Effects of ingredients and processing on analogue cheese attributes are outlined.

ABSTRACT

Background: Analogue cheeses (AC) are homogeneous cheese-like matrices obtained by mixing water, oils/fats, proteins, emulsifying salts and other ingredients under the influence of heat and mechanical shear. These versatile products are used both directly for consumption and mainly indirectly as ingredients in several foods.

Scope and approach: Increasing consumers' expectations, consumption habits, current lifestyles and cheese industry dynamism are factors driving the research towards the development of new cheese-like products and functionalities. This review describes the state of the art on AC formulations in relation to properties of the final product.

Key findings and conclusions: Extensive data from research on AC highlight the current growing interest for the development of innovative functionalities to satisfy specific end-use applications. The outcomes of most investigations drew attention to the basic role of type and amount of ingredients to obtain a wide array of customized attributes. An insight on the role and the interactions among constituents of the formulation and the effect on textural, rheological, melting and sensory properties of AC was provided.

STATUS AND DEVELOPMENTS IN ANALOGUE CHEESE FORMULATIONS AND FUNCTIONALITIES

Fabio Masotti*, Stefano Cattaneo, Milda Stuknytė, Ivano De Noni

Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Università degli Studi di Milano, Via G. Celoria 2, 20133 Milan, Italy

*Corresponding author. Tel.: +39 0250316665; fax: +39 0250316672.

E-mail address: fabio.masotti@unimi.it (F. Masotti).

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Background: Analogue cheeses (AC) are homogeneous cheese-like matrices obtained by mixing water, oils/fats, proteins, emulsifying salts and other ingredients under the influence of heat and mechanical shear. These versatile products are used both directly for consumption and mainly indirectly as ingredients in several foods.

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Keywords: Analogue cheese; Dry ingredients; Casein; Emulsifying salts; Functional properties.

Definitions and applications

The global production of dairy products is constantly growing over time. In particular, within the group of processed dairy products, cheese consumption is expected to continue to account for the greatest share at an annual average rate of 1.6% (OECD/FAO, 2015). The projections of overall cheese market outline a steady growth ascribable to different factors: (i) the change in consumers' life-style, (ii) the innovation in cheese types and their versatility, (iii) the broadening of applications of cheese in our diet and as an ingredient in food processing (Chandan & Kapoor, 2011; Guinee, 2016). In this context, the market of cheese-like products is expanding faster than that of natural cheeses. An increase in the production of new varieties including AC, fulfilling the growing demand in developing dairy markets, is expected (PMFood & Dairy Consulting, 2014). These products are categorized in analogue cheeses (AC) and fat-filled cheeses, the former one dominating over the other. Analogue cheeses is the term generally referred to products obtained by mixing water, oils/fats, proteins, emulsifying salts (ES), starch, hydrocolloids, acidifying agents, preservatives and other food additives in a homogeneous cheese-like matrix.

Analogue cheeses are systematically grouped into dairy, partial dairy or non-dairy, depending on the origin of the ingredients used in their formulation. By far, partial dairy AC, in which the proteins are dairy-based and the fat is mainly of vegetable origin, are the most common (Guinee, 2016). In comparison to natural cheeses, this group of AC is cost saving due to the use of cheaper vegetable oils instead of milk fat and the partial substitution of protein by starch and/or other hydrocolloids. Cost-effectiveness is also attributable to the lower capital cost of manufacturing equipment and the fact that no ripening is required (Guinee, 2011). AC are amenable to incorporate valued functional ingredients. They show relatively stable textural, flavor and cooking properties during cold storage. Additional advantages of AC lies in compositional and nutritional flexibility, health and dietary benefits, tailored manufacture to customize functionalities (textural, cooking) and finally convenient packing and ease-of-use. Non-dairy AC (largely obtained from vegetable proteins and fats) represent a market opportunity for vegan cheeses. Generally, the substitution of casein (CN) by vegetable proteins has resulted in AC with impaired texture

(Fox, Guinee, Cogan, & McSweeney, 2017a). Dairy AC are produced in low quantities, as their manufacturing costs are higher than those of natural cheeses owing to the extra cost associated with the preparation and reconstitution of ingredients (Fox et al., 2017a). At global level, the definition of these cheese-like products is not uniform, and clearer legislation is required to warrant proper labelling and to avoid deception of the consumer. Codex Alimentarius (2016) defined AC as products that look like cheese, but in which milk fat has been partly or completely replaced by other fats. In the US specific legislation covers AC. Indeed, regulations from the Food and Drug Administration classify AC as imitation cheeses (when they substitute and resemble a cheese but are nutritionally inferior to the natural counterpart cheese) and cheese substitutes (when they are not nutritionally inferior) (Guinee, 2007). In both cases, they differ from natural cheese varieties, not complying with Codex Alimentarius definitions (Codex Alimentarius, 2000) or national rules.

The US gave birth to AC more than 40 years ago (Fox et al., 2017a). Generally, at the beginning of their invention, imitation foods were considered a deviation from the conventional counterpart (Williams & Carter, 2011). In other words, they were regarded as simple mimicking of the conventional food for its replacement in humans' diet. Anyway, over time AC production was supported and addressed mainly to obtain cheaper and healthier alternatives, to increase diet variety and to fulfill consumers' expectations in specific end-use applications. Other positive aspects distinguish AC from its natural counterpart, i.e. relatively stable texture, flavor and cooking properties during cold storage, health and dietary benefits, compositional and nutritional flexibility, employment of different functional ingredients, tailored manufacture to customize functionalities, convenient packaging and ease-of-use. Such positive aspects made the success of AC. Nowadays, the market of AC thrives in the US, which represents the major producer region (Fox, Guinee, Cogan, & McSweeney, 2017b). Substitutes for or imitation of low-moisture Mozzarella (MC), Cheddar, Monterey Jack and pasteurized processed Cheddar (Guinee, 2007) are the major AC varieties. Prevailing sectors of delivery of AC are represented by: (i) the retail chain (directly as table products, through stores or direct-to-consumer markets) (e.g., frozen pizza, cheese-filled ravioli, dried pasta dishes and cheesecake), (ii) the food service sector, as ingredient in dishes (e.g.

lasagne, pizza, omelette, cheese panini and salad dishes) and (iii) the industrial sector as ingredient in assembled food products, co-extruded foods, formulated foods, etc. (Fox et al., 2017b). Applications of AC are both in the unheated (cheesecake, desserts, salads, snacks) and heated state (pasta dishes, Pizza, sauces). To meet the constantly increasing consumers' demand, a major requisite of AC consists of guaranteeing the end use performance of the cheese product.

Data on current annual manufacture of AC are scarce. The US is the major producer (~ 3×10^5 t per year) of AC, whereas smaller production is estimated in Europe (2.0×10^4 t), likely due to the lower use of cheese as an ingredient in formulated foods (Fox et al., 2017a). By considering the consumer changes in food habits, we judge timely and worthwhile to present an overview of the current literature on AC processing, emphasizing the role of ingredients, criteria used in their selection and developments of formulations customized to specific end-product functionalities.

Analogue cheese properties

Specific attributes are required to AC to perform the desired end-use application (Guinee, 2016). The functional characteristics of AC, both when directly consumed and when used as ingredient, play a major role for its successful acceptance by the consumers/food service providers. The functionality of AC is influenced by micro- and macro-structure, which in turn are affected by factors such as processing conditions and composition of the AC (Fox et al., 2017b) (Fig. 1). Type (fat, CN, whey proteins, ES) and physico-chemical properties (degree of protein aggregation or hydration, size distribution of emulsified oil droplets) of ingredients and interrelations among components (protein-to-fat ratio, calcium-to-CN ratio) are major determinants of rheology and functionalities of the end product. Unheated AC, when used as ingredient cheese, may be required to exhibit one or more functional attributes such as the ease of spreading, crumbliness, sliceability and shreddability. In addition, AC should have defined organoleptic (i.e. flavor and texture) properties both in direct (during mastication) and indirect consumption (as an ingredient) (Guinee, 2016). Cooking properties of AC are a composite of different functionalities, including softening, flowability, tendency to brown, oiling off and/or stretchability (the latter for pasta filata varieties) (Guinee, 2011) (Fig. 1). Both unheated and heat-induced functionalities are strictly related to physical (rheological) characteristics (hardness, elasticity, adhesiveness, brittleness or crumbliness) of AC (Guinee, 2016) (Fig. 1).

Ingredients of AC: role and effects on functionalities

The formulation of AC consists of a combination of proteins, fats/oils and water as key ingredients. Other ingredients include ES, hydrocolloids, acidifying agents, flavors and preservatives (Guinee, 2007; Jana & Upadhyay, 2001; Jana, Upadhyay, & Solanky, 2005). Generally, dry ingredients (apart from fats/oils) adequately mixed together in water, under heat and mechanical shear, give an oil-in-water emulsion resulting in a homogeneous cheese-like mass. Most formulations of AC are patented, likewise manufacturing protocols. Some examples of AC formulations reported in literature are presented in Table 1. It is noteworthy the wide array of dairy protein sources exploited in these trials. Generally, the variability in AC formulation (coupled to differences in manufacturing procedures) is reflected in the large diversity in offered functionalities (e.g. flowability, melt resistance) and their capability to satisfy specific dietary needs or benefits/supplements (Guinee, Caríc, & Kaláb, 2004). The aim of most ongoing investigations consists in assessing the impact of type and amount of components in the blend on customized end-use applications.

Water

Water represents the major ingredient of AC with average values ranging from 48% to 52%. Besides water as an ingredient, the final moisture of AC is attributable also to condensate when the blend is cooked by direct steam injection (Fox et al., 2017b). Noronha, O'Riordan, and O'Sullivan (2007) in laboratory-made AC reported moisture levels up to 60%. These AC resulted soft or tough as a function of the amount of resistant starch added. Water is necessarily used to dissolve ES, to hydrate proteins and to solubilize other ingredients. In general, the impact of water consists in decreasing the hardness and increasing the meltability of AC. The hydration of high-protein dairy powders is essential in AC manufacture and it is an item of utmost importance at industrial level. The functionality of a CN-based powder is affected by its physico-chemical properties (e.g. hydration/solubility, foaming and emulsifying properties), which in turn depend on parameters such as selection of raw material, pre-treatments (changes in pH, ionic strength), method of preparation and storage conditions (O'Sullivan, Singh, Munro, & Mulvihill, 2002). During mixing of ingredients, water helps in dissolving the ES, resulting in the better chelation of calcium, the disruption of the structural integrity of CN and its ensuing solubilization (Lee, Anema, & Klostermeyer, 2004). In the final cooling of AC production, entrapped water molecules act as a plasticizer, making the protein network less elastic and more susceptible to fracture upon compression (Fox et al., 2017b). Ennis and Mulvihill (1999) reported that an extensive protein hydration increased the emulsifying ability of proteins, allowing the formation of a greater oil phase surface, and ultimately improving the stabilization of the dispersed oil phase in cheese matrix. The authors concluded that to increase the water content of AC (as a tool to reduce costs), proper disruption and hydration of the rennet CN structure is necessary, and this result is obtained through an adequate choice of ES mixture (see also the section Emulsifying salts). An excess of water, promoting extensive over-hydration of protein, could cause the phenomenon of over-emulsification resulting in unsatisfactory performances of AC (i.e. poor meltability and reduced flow upon heating).

Proteins

In addition to water and fat, proteins represent a foremost ingredient of AC, with typical levels ranging in the formulation from 18% to 24% (Guinee, 2007). Arimi, Duggan, O'Sullivan, Lyng, and O'Riordan (2012) reported a content of protein in AC up to 35%. The authors developed a microwaveable snack food characterized by low fat content in comparison to fried snacks. This imitation cheese-type product, is essentially a protein-based matrix containing resistant starch, expanding when microwaved to form a crispy product. The authors observed that increasing protein content in AC from 14% to 35% was paralleled by an increase in the volumetric expansion values from 390% to 440% after microwave heating. This phenomenon was explained by the fact that it is the hydrated protein matrix alone that stretches and swells under the pressure of steam during microwaving.

Several investigations (Mounsey & O'Riordan, 2008a, 2008b, 2008c) described the feasibility to reduce protein level as a tool to reduce costs, compensating the modified functionalities by starch replacement (Table 2). Kiziloz, Cumhur, and Kilic (2009) by replacing 80% rennet CN with partially hydrolyzed waxy maize starch and κ-carrageenan developed an AC (with textural properties similar to those of the high protein counterpart). In details, trial samples contained 5% rennet CN, 20% butter, 22.0–24.5% waxy maize starch, 0.5–3% k-carrageenan, 0.5% NaCl, 0.3% citric acid, 0.22% trisodium citrate, 0.1% disodium phosphate and water. Starch was partially hydrolyzed by α-amylase to obtain a gummy fluid structure leading to softening of the AC matrix (in comparison to the control AC containing 25% rennet CN) and contributing to its meltability upon baking. k-carrageenan in interaction with rennet CN formed the network with partially hydrolyzed amylopectin, fat and water entrapped in the structure. Interactions increased as the concentration of k-carrageenan was increased, thus strengthening the network and reducing meltability.

During AC manufacture, proteins are the main ingredient that hydrates. The solubilized proteins emulsify the free oil and increase the viscosity of the aqueous phase (Mounsey, 2009). The droplets of dispersed oil are stabilized and a fat-entrapping network is formed. Type, amount, structure, mineral content and solubility of proteins exert a remarkable influence on physicochemical, rheological, stability and cooking properties of AC (Sołowiej, Cheung, & Li-Chan, 2014; Guinee, 2016). A large variety of protein sources is exploitable in AC production, and those of dairy origin are preferred. In particular, rennet CN and caseinates prevail for their inherent functionalities (e.g. hydration, fat emulsification). Literature reported also that acid CN and rennet CN are characterized by a dirty brothy/animal flavor (Karagül-Yüceer, Vlahovich, Drake, & Cadwallader, 2003; Smith, Campbell, & Drake, 2016). Caseins find an important outlet in AC manufacture, especially in Pizza cheese (Fox, 2001). Acid CN and rennet CN differ significantly in composition. In the first one the micellar calcium and phosphate are depleted during the acidification step of manufacture. Rennet CN is characterized both by the high colloidal calcium content and the loss of the hydrophilic C-terminal of κ-CN (i.e. caseino-macropeptide), which is responsible for the lower water binding capacity in comparison to acid CN (Sołowiej et al., 2014). The solubilization of rennet CN powder is reached by adequate processing conditions and the choice of an ES mixture (typically citrates and phosphates) to obtain a *para*-caseinate matrix (El-Bakry, Duggan, O'Riordan, & O'Sullivan, 2011b) (See also the section Emulsifying salts). Generally, acid CN is industrially used up to levels of 1–3%, due to its scarce solubility and the low pH, which reduces the ability of ES in the mixture to sequester calcium from other proteins namely rennet CN (Guinee, 2011). Anyway, acid CN is reported to hydrate better than rennet CN when converted to sodium or calcium caseinate by alkalinization because its water binding capacity is enhanced (Guinee, 2011). Some authors (Gustaw & Mleko, 2007; Sołowiej et al., 2015) reported the suitability of acid CN as the major protein source (10−13% w/w in the formulation) in the manufacture of processed cheese analogues (See also sections Hydrocolloids and Preservatives).

The use of rennet CN prevails over other protein sources especially in Mozzarella cheese analogue (MCA) (at rates of ~ 18−24%) (see also Table 1). Rennet CN confers a high degree of elasticity and firmness to the unheated MCA and moderate meltability upon baking (Guinee, 2009). Rennet CN also contributes to the stretchability of the hot mass during the manufacture of MCA for Pizza pie. The amount of rennet CN used in formulation plays an important role on the final properties of AC. Jana, Shah, Aparnathi, and Padhiyar (2015) evaluated the functionality and sensory quality of MCA samples for Pizza topping obtained using amounts of 22.0%, 24.5% or 27.0% of rennet CN in the formulation. The MCA sample with 24.5% rennet CN was preferred for the meltability (Schrieber test), the moderate fat leakage and the adequate stretchability. Sensory analysis carried out through hedonic tests measured appearance, flavor, melting and chewiness attributes of AC. The panel of assessors significantly preferred the MCA sample with 22.0% rennet CN, whereas the sample obtained with 27.0% CN received the least scores.

Sodium caseinate as the only protein source was reported to be undesirable for Pizza topping, because the derived AC showed poor meltability and firmness (O'Riordan, Duggan, O'Sullivan, &

Noronha, 2011). Caseinates (especially sodium caseinate) are preferred over CN especially in spreadable imitation cheese products (Guinee, 2009) because of their high water-binding capacity and good emulsifying properties capable to promote a desired creaming effect. The use of other dairy derivatives or the combination of different protein sources were useful strategies to pursue specific functionalities and received a deal of attention in past years. For instance, the partial substitution of sodium caseinate (90% protein) with the same amount of whey protein concentrate (WPC, 35% protein) (at rate of 1.5%) caused slight increase in AC hardness and a reduction of both meltability and sensory properties (measured by hedonic ratings of untrained panellists) in comparison to sodium caseinate-based AC (Hosseini, Habibi Najafi, & Mohebbi, 2014). The trial AC sample was slightly lower in protein content in comparison to the control (21.4% vs 22.2%). The negative effects on sensory properties were offset by addition of 0.3% guar gum in the formulation.

Whey proteins (WP) derivatives (typically WPC), as the alternative to CN for nutritional and economic reasons, exert complex effects on the properties of AC. Several factors (degree of denaturation of WP, composition of AC and processing conditions) affect overall properties of AC utilizing the WP source. Typically, the lower amphiphilic behavior of WP in comparison to CN promotes a reduced emulsifying capability of free fat (Guinee, 2009). A limited addition of whey-based derivatives (typically WPC), restricted from 1% to 3% of the formulation of rennet CN-based AC, was suggested in those applications where flow-resistance is envisaged (e.g. burgers) (Guinee, 2011). Generally, the addition of WP derivatives increases hardness and decreases meltability. Dhanraj et al. (2017) studied the influence of WPC as partial rennet CN replacer in the formulation of MCA used as pizza topping. Blends of rennet CN:WPC (68% protein) at ratios 95:15, 90:10, 95:5 were evaluated in terms of baking and sensory properties. MCA samples of different protein blends showed only slight differences in terms of shredding, meltability (Schrieber test), stretch test (Fork test) and total sensory scores (hedonic tests evaluating appearance, flavor, melting, and chewiness attributes). The sample with protein blend at CN:WPC ratio 90:10 was slightly preferred over the other blends. Sołowiej et al. (2014) focused on the influence of increasing amounts (from 1% to 3%) of high-protein derived products (i.e., WPI at 94% protein or WPC at 80% protein) as protein replacers in a processed cheese analogue obtained from rennet CN (at protein rates ranging from 10% to 13%). In comparison to samples obtained using rennet CN alone, AC with added WP derivatives showed progressive increase in hardness, adhesiveness and viscosity, while meltability decreased. Analogue cheeses with WP derivatives were good for shredding, but with poorer melting properties. These results pointed out that the use of the appropriate combination of CN and WP is a tool to control textural/rheological properties and meltability of AC.

The addition of WPC (80% protein) solutions at different degrees of denaturation (by heating at temperatures from 67 °C to 85 °C for holding times of 150 s in a scraped-surface exchanger) in the formulation affected the rheology and microstructure of rennet CN-based spreadable AC (Lee, Huss, Klostermeyer, & Anema, 2013). Degrees of denaturation in AC with microparticulated aggregates ranged from 40% to 100%. Higher levels of WP denaturation in AC caused a decrease in hardness and an increase in the meltability of the resultant spreadable AC compared to samples with unheated WPC solution. This effect was attributed to the incorporation of denatured WP as relatively large particles that did not contribute to the matrix structure, thus disrupting the AC network. In contrast, following the addition of native WP, the manufacturing conditions of AC promoted a only partial denaturation (~ 40%) of WP resulting in their subsequent incorporation into the matrix, producing a stronger structure.

Due to the high cost and some functionality limitations of CN (e.g. limited hydration capacity and solubility in water), several studies, mostly dating back in the 1980s and 1990s, were undertaken to evaluate the use of more affordable vegetable proteins (Bachmann, 2001). Peanut, cottonseed and soy protein isolates alone or in blend with CN showed a limited success, due to the inferior quality of AC when these ingredients were used instead of CN (Fox et al., 2017a; Mounsey & O'Riordan, 2001). Generally, vegetable proteins at substitution levels higher than 20% cause texture problems, namely lack of elasticity, reduced hardness, sticky body, reduced stretchability often associated with impaired flavor (Guinee, 2016; Chavan & Jana, 2007).

Fats

Typically, fat content varies from 22% to 28% and contributes to both flavor and texture of AC (Mulsow, Jaros, & Rohm, 2007). At room temperature, about 15% of the milkfat should still be a solid forming an exterior crystalline layer, while the oil in the interior of the fat globules. The solid component consists of a network of triglyceride crystals connected by Van der Waals forces of attraction. Following ES addition, shearing and heating actions during AC manufacture, the rupture of these bonds takes place, forming melted oil droplets of variable diameter, which are emulsified by the protein (Lobato-Calleros, Vernon-Carter, & Hornelas-Uribe, 1998). The size distribution of emulsified oil droplets together with the degree of *para*-caseinate hydration are major determinants of the rheology and heat-induced functionality of the AC (Guinee, 2016). The droplet size decreases as emulsification intensity increases, and as processing conditions become more intensive. *Para*-caseinate-coated oil droplets interact with casein strands and contribute to the homogeneous network (Mulsow et al., 2007). In dairy based AC, the fat component is of dairy origin, i.e. anhydrous milk fat, butter, or cream. Conversely, in AC categorized as partial dairy, the oil/fat source may be milk fat in addition to vegetable oil/fat. Typical fat sources are soy oil, palm oil, rapeseed oil or their hydrogenated corresponding fats (Guinee, 2016). Functional benefits, cost-cutting and/or health claims (absence of cholesterol in non-dairy AC and reduced levels of saturated fatty acids) account for replacement with vegetable sources. To date, the use of vegetable oils appears well established, although few studies on the effect of partial/total milk fat substitution with different vegetable sources (soybean, peanut, palm kernel, corn or coconut) are available (Lobato-Calleros et al., 1998; Budiman, Stroshine, & Campanella, 2000). The common result of these investigations is that different types of oils have different fatty acid composition, which in turn affects physicochemical characteristics of oil thus influencing the properties of AC (Mulsow et al., 2007). Generally, the total or partial replacement of milk fat by vegetable oils (up to 50%) (liquid at room temperature) resulted in softer AC (Budiman, et al., 2000; Cunha, Dias, & Viotto, 2010; Shabani, Sarfarazi, Mirzaei, & Jafari, 2016), while the use in the formulation of vegetable fats resulted in harder or similar consistency AC in comparison to that of butter. To elevate the melting point of a vegetable oil to a level similar to that of milk fat (32−34 °C), the former is required to be partly hydrogenated or suitably modified (interesterification). Cunha, Grimaldi, Alcântara, and Viotto (2013) showed that partially hydrogenated soybean fat (melting point of 36 °C) in substitution to butter oil (melting point of 34 °C) resulted in a spreadable analogue cheese with lower size of fat globules and greater hardness. Lobato-Calleros et al. (1998) found that AC formulations containing soybean fat (melting of point of 35−38 °C) had higher hardness than the AC made with 100% butterfat (melting point of 32−34 °C). Indeed, the texture of AC is affected by the structural arrangement of components (proteins and fat/oil), which in turn are related to factors such as composition and processing (Fig. 1). Fat droplet size exerts a significant role in structure of the AC matrix (Lobato-Calleros et al., 1998). The size of free fat droplets varies a lot, decreasing as shearing action becomes more vigorous and emulsification increases. *Para*-caseinate-coated fat globules connected to the matrix strands contribute to the continuity of the gel network structure (Mulsow et al., 2007). Hanáková, Buňka, Pavlínek, Hudečkhová, and Janiš (2013) studied the influence of some fats/oils (butter, coconut fat or palm oil) on the textural properties of AC. As expected, the rising proportion of saturated fatty acids (with higher melting point) in the fat phase paralleled the increase of hardness values in AC (palm oil < butter < coconut fat). The content of fat in AC, in particular in MCA, generally ranged from 22.0% to 29.9% (Guinee, 2011). An item of interest for manufacturers since the 1990s has been the production of new cheese products low in fat. Fat reduction causes an increase in the protein network and a decrease in lubrication upon melting, resulting in a hard and crumbly texture which is undesirable from a sensory perspective. The adverse effects of a high concentration of protein on the properties of low-fat AC may be attenuated by modifications of the manufacturing process to reduce the levels of calcium-to-CN ratio and by increasing the levels of moisture. Increased hydration has a plasticizing effect (Noronha 2007) (See also the section Starch).

Emulsifying salts

Emulsifying salts do not act in AC as true emulsifiers, being not active surfactants. They promote hydration and solubilization of proteins by triggering several physico-chemical changes in the cheese-like matrix. Generally, their content in the formulation ranges from 0.5% to 3% (w/w) (Guinee, 2011). Types and properties of different ES have been reviewed (Lucey, Maurer-Rothmann, & Kaliappan, 2011). Various ES are used in AC including citrates, lactates, phosphates and tartrates, with ammonium, potassium or sodium as a counter-ion. Typically, a mixture of phosphates (disodium phosphate) and citrates (trisodium citrate) in the ratio of 1:1 is used in AC manufacture (El-Bakry et al., 2011b). Typical levels of ES used in formulation of AC are reported in Table 1. During AC processing, ES influence a complex sequence of actions, namely calcium sequestering, increase of pH value, stabilization (buffering) of pH level, protein dispersion and hydration, fat emulsification and structure formation. In details, their addition to rennet CN causes the exchange of colloidal calcium in the *para*-CN network for sodium of ES. Consequently, the insoluble protein network is partially solubilized to sodium *para*-caseinate dispersion. This action of ES is coupled to an increase in the pH, which in turn assists further protein dispersion and hydration (El-Bakry et al., 2011c). The new conformation of *para*-caseinate dispersion enhances its emulsifying ability. Types and ratios of ES in blend can affect the ability to sequester calcium and consequently protein hydration and fat emulsification. El-Bakry et al. (2011b) verified the influence of different ratios of a disodium-phosphate:trisodium-citrate blend, from 0:1 to 1:0 in the manufacture of a 50% moisture rennet CN-based AC. The authors verified that changes from the standard ES ratio (1:1) modified the hardness of the final product. By increasing phosphate:citrate ratio from 1:1 up to 1:0, the mean fat globule size in AC increased from 20 μm to 31.5 μm and the cheese matrix was softened. Under these conditions, protein-protein interactions were supposed to be less disrupted due to low calcium solubilization. The consequent lower hydrated CN resulted in reduced fat emulsification, ultimately leading to a less plasticized matrix. Differently, the cheese made with citrate as a sole ES had the smallest fat globule size (7.6 μm) and highest hardness. Thus, altering the ratio in the ES blend may allow tailoring AC properties for specific end uses. For instance, cheese can be softened by increasing disodium-phosphate:trisodium-citrate ratio, e.g. in case of reduced fat AC.

McIntyre, O'Sullivan, and O'Riordan (2016) evaluated the effect of different types (disodium phosphate and trisodium citrate), concentrations (10 and 30 mmol L^{-1}) and ratios (1:0, 2:1, 1:1, 1:2 and 0:1) of ES in rennet CN dispersions (15%), under pH (6.7) and temperature (70 °C) simulating industrial processing of AC. Disodium phosphate was weaker at solubilizing calcium than trisodium citrate, allowing only partial disruption of the calcium crosslinks in the rennet CN, though sufficient to form a hydrated matrix. Independently of the ratio of calcium chelating salts in the blend, the level of solubilized proteins was low (max 11% of total proteins). In details, in ES-free CN dispersions the solubilized proteins were 2.2% (on total protein), whereas following addition of phosphate from 10 to 30 mmol L^{-1} increased the protein content in the dispersed phase only up to 3.1%. Differently, the use of citrate resulted in a greater level of protein solubilization (5.4% and 11.6% of total protein for 10 and 30 mmol L^{-1}, respectively). The use of intermediate ES blends (at ratios 2:1, 1:1 or 1:2) showed that higher proportions of phosphate (with respect to citrate) resulted in lower amounts of protein in the dispersed phase (2.9–7.3% of total protein) in comparison to matrices obtained using only citrate. The authors hypothesized that only a low level of soluble proteins is required to emulsify fat, due to the high emulsifying capacity of CN. They concluded on the need of further investigations to understand the role of ES in modulating hydration during manufacture of CN-based matrices, being more complex than simply solubilizing calcium or proteins.

In recent years, the reduction of sodium in processed foods is an issue of ongoing concern (Table 2). El-Bakry, Duggan, O'Riordan, and O'Sullivan (2010) investigated the impact of decreasing sodium in AC, through reduction of ES concentration in the formulation from a standard level of 1.4% up to about 0.8%. In the standard AC the ES mixture consisted of trisodium citrate and disodium phosphate at a ratio of 2.16:1. Over the range of ES reductions, a progressive increase in processing time and in the mixing energy needed to emulsify the fat phase. As a consequence, a decrease of fat globule diameter was observed. The prolonged mixing during manufacture increased hardness and decreased the heat-induced flowability. Only a ES reduction of up to 20% was judged feasible, being above AC functionalities only slightly altered and processing times increased by only 25 %. A reduction of sodium content was achievable also by the replacement of sodium ES blend (disodium phosphate and trisodium citrate, at ratio 0.46:1.0) with their potassium equivalents (El-Bakry et al., 2011a) in a 48% moisture rennet-based AC. This substitution led to better protein hydration which required less mixing energy of AC ingredients.

Adding potassium-ES (instead of sodium-ES) resulted in a slight increase in pH value (from 6.0 to 6.3) due to less potassium binding (with respect to sodium) to CN and to a less displacement effect of hydrogen ions of CN. The increased pH was hypothesized to increase electrostatic repulsion between the CN molecules, thus improving protein hydration and, as a consequence better fat emulsification. Differences were observed during refrigerated storage in functional properties. The increases in pH value and fat globule size promoted slight decrease in hardness, a less cohesive structure and slight increase in flowability and adhesiveness. The increase in pH value of AC obtained with potassium ES was hypothesized as the cause of their reduced microbial stability during 12 weeks storage (at 4 °C) in comparison to the sodium equivalents. The trial was carried out at laboratory-scale and thus under hygienic conditions not as strict as in industrial processing (i.e. under controlled air flow and using aseptic packaging). After manufacture, the samples with sodium ES (pH 6.0) showed a total viable count of ~ 10^1 ufc g^{-1}, which slightly increases up to 10^2 cfu g^{-1} throughout the storage period. The substitution of the sodium salts by their potassium equivalents led to substantial higher counts (up to10^4 cfu g^{-1}). In either formulations no mould or yeast growth was detected.

Starch

The inclusion of starch in AC formulation modifies physico-chemical and functional attributes of the final product to an extent related to starch properties, such as type, shape, size, swelling ability and the amylose/amylopectin ratio affected the physico-chemical properties of AC (Mounsey & O'Riordan, 2001; Guinee et al., 2004; Ye, Hewitt, & Taylor, 2009). Noronha, Duggan, Ziegler, O'Riordan, and O'Sullivan (2008c) investigated the inclusion of 1.9% up to 9.9% of starch (native, pre-gelatinized, resistant or waxy corn) in a 55% moisture AC. Increasing the starch content changed the texture of cheeses from ‘soft’ to ‘tough’ and decreased the mobility of water. Starches with different gelling properties are exploitable in meeting specific needs, i.e. less melting AC suitable for applications in which flow resistance is required. Native and pre-gelatinized starches became swollen and separated protein and starch phases. Resistant and waxy corn starches resulted unswollen and occurred in small discrete particles and unchanged by the cheese manufacturing process.

Native starch is added to AC formulations at levels of 2–4% to substitute ~ 10–15% rennet CN for its relatively low cost, availability and benefits (Considine et al., 2011; Guinee, 2011). Commercially native maize starch has been reported as the main type used (Guinee, 2011). Several investigations were carried out to evaluate the effect of both native and modified starches of different botanical origins as partial CN replacer (Mounsey & O'Riordan, 2008a, 2008b, 2008c, 2008d; Sołowiej et al., 2016). The partial replacement (15%) of rennet CN with wheat, maize, waxy-maize or rice starches resulted in AC with fat globule diameters (2–10 μm) smaller than the starch-free control, thus indicating more extensive fat emulsification (Mounsey & O'Riordan, 2001). The authors observed that during AC manufacture the oil of starch-containing AC was emulsified more rapidly than the control. The starch likely behaved as a filler in the early, low-temperature stages of AC manufacture, leading to increased availability of water to hydrate the CN, and subsequently improved fat emulsification. An important impact of partial substitution of rennet CN with starch was the reduction in AC meltability in the order maize > waxy-maize > wheat > rice (Mounsey & O'Riordan, 2001, 2008d). This effect was attributed to different factors such as fat globule size and the disruption of protein matrix by swollen starch granules. The latter favoring the immobilization of water by the granules may have resulted in the dehydration of the protein matrix, which led to increased hydrophobic protein-protein interactions and ultimately poorer meltability. The protein dehydration was likely responsible of a honeycomb structure of the protein matrix in the area immediately adjacent to the starch structure. Rice starch with its small granule diameter, relatively low amylose content (13% *vs* 25% and 28% of maize and wheat, respectively) and small swelling capacity had the least effect on melting reduction, resulting in an AC with acceptable rheological properties. Mounsey and O'Riordan (2008a) reported that when native wheat starch completely replaced rennet CN, obtained AC was non-melting-type. Starches with high amylose content (25% maize, 28% wheat) resulted in reduced flowability of the melted AC owing to retrogradation (Mounsey & O'Riordan, 2001, 2008d). Pre-gelatinized starch, differently from native, is dispersible in cold water. As a consequence, when the former is used in AC it is more likely to interact with the protein network rather than being a filler. Pre-gelatinized starches (of maize, waxy-maize, wheat, potato or rice) were used at rates of 3% as a replacer of 15% rennet CN in the formulation of AC (with 17% protein *vs* 20% protein of the starch-free control) (Mounsey & O'Riordan, 2008b). Analogue cheeses with above pre-gelatinized starches showed poorer fat emulsification resulting in softer, less cohesive AC that had reduced meltability, in comparison to the control AC. The inclusion of pre-gelatinized starch in AC would only be useful when low meltability in a heated system is required (deep fried cheese) or in a ‘‘cold” application (salad bar) (Mounsey & O'Riordan, 2008c).

Resistant starch was investigated as a source of dietary fiber to provide AC with the image of a health promoting and more nutritious food. Noronha et al. (2007) in an AC with 52% moisture and 12% fat contents replaced about 50% fat, consisting of hydrogenated palm oil and rapeseed oil (in the ratio 2:1), with native resistant starch. The observed excessive hardening effect was reduced by increasing the moisture of AC to 60%. The authors verified that at this moisture level the further replacement of up to 90% of fat with starch allowed to obtain a 2% fat AC with hardness and cohesion values amenable to slicing, shredding and moulding. This research demonstrated that a high moisture content (60%) plasticized AC matrix allowing the cheese to melt even if it contained only 2% fat. After sensory analysis by ranking tests, this high-moisture low-fat AC lost its grainy texture and panellists perceived it as smoother and firmer in comparison to samples where up to 85% fat content was replaced. The improved mouthfeel was attributed to the fact that more abundant and smaller starch particles in the 2% fat cheese may not have absorbed as much water as particles in the cheeses containing lower levels of starch.

Low-fat starch-containing AC have the potential to expand during microwave heating, thus representing a novel way of producing crispy snack foods (Arimi, Duggan, O'Sullivan, Lyng, & O'Riordan, 2011). These products stand out for reduced preparation time and low fat in comparison to fried snacks (Arimi, Duggan, O'Sullivan, Lyng, & O'Riordan, 2010). The expansion process is mainly influenced by moisture content. These authors verified that hardness, elastic (G') and viscous (G″) moduli increased with decreasing moisture content (from 60% to 45%) of AC. The decrease in moisture content resulted in a decrease in the volume of microwaved expanded AC (from 190% to 160%). Moisture during microwave turns into steam providing the pressure for expansion and, at the same time, it plasticizes the matrix allowing the expansion to occur (Arimi et al., 2012). The resistant starch used, i.e. high amylose corn starch has a low water-holding capacity and does not swell during microwaving due to the insufficient water in the vicinity of the granules as most of it evaporates quickly due to the rapid nature of microwave heating. Moisture exerts a primary role also during pre-expansion cold storage time. The authors observed (by nuclear magnetic resonance relaxometry) that tightly bound water decreased during AC cold storage (up to 9 days). Such phenomenon was attributed to an increase in water mobility. The more mobile water increased the plasticization of the AC matrix making it more stretchable under pressure during microwaving. After 9 days storage the volumetric expansion of 60% moisture AC increased about 4-fold in comparison to 45% moisture AC.

Inulin

Inulin is a widespread ingredient used in dairy products with multiple purposes: dietary fiber, sweetener (when partially hydrolyzed) and to improve organoleptic properties. In cheese, it is mostly applied both as fat replacer, due to its rheological properties similar to table fats, and as texture modifier (Karimi et al., 2015). The simplest method of incorporation in AC would be as dry powder, but in this way its inclusion would lead to hard lumps and free water. To improve the distribution of inulin in AC, Hennelly et al. (2006) studied the effect of incorporating inulin either as a heated solution (80 °C) or as a shear-induced gel to replace 63% hydrogenated palm oil in a 54% moisture AC. The fat content was 11.0% in AC samples with 3.4% inulin, while in the control sample (inulin-free) was 21.7%. As a result of fat replacement, rennet CN content passed from 20.1% in the control sample to 23.8% in AC containing inulin. The inulin incorporation as a gel or as a solution had no significant effect in terms of AC microstructure and meltability in comparison to the control sample. The presence of inulin in AC caused a significant increase in hardness in comparison to inulin-free samples. Such result was apparently unexpected because hydrogenated palm oil is a hard fat (melting point 48 °C). Its substitution with a soft inulin gel or solution would be expected to result in a decrease in hardness. Being unchanged AC moisture level (54%), this virtual softening was more than offset by the increase of protein:fat and protein:moisture ratios of inulin samples with respect to control sample. In acid CN-based (10%) processed cheese analogues (13% protein, 30% fat contents) added with WP polymers (1–3%), Sołowiej et al. (2015) investigated the effect of the partial replacement of anhydrous milk fat (5–15%) with inulin (1–3%). Modifications in AC consisted in increased meltability (with 2–3% inulin) and decreased hardness (with 1% inulin) and adhesiveness (with 3% inulin) in comparison with full-fat (control) samples. The authors suggested that above effects in the CN-WP polymers-inulin/fat matrix could be exploited in low-fat processed analogue cheeses to offset the undesired modifications of fat reduction.

Hydrocolloids

Hydrocolloids together with ES are classified as stabilizers in the literature. Apart starches, a wide variety of polysaccharide-based hydrocolloids are included in AC formulation, commonly in the manufacture of high-moisture and low protein AC (Guinee, 2011). Despite the addition is as little as up to 0.3% (Guinee, 2007), the texture and the stability of AC are modified. Hydrocolloids have water binding and stabilizing properties and contribute to the reduction of the energy value of AC (Sołowiej & Nastaj, 2016). Polysaccharide-based hydrocolloids used in AC manufacture are categorized in neutral gums (e.g., agar combination, basil seed, guar, konjac glucomannan, locust bean,) and anionic gums (e.g., alginates, pectins, κ-carrageenans, ι-carrageenans, λ-carrageenans and xanthan). Typically, these gums assist in moisture management favoring desirable texture and AC stability (Chandan & Kapoor, 2011). Several studies evaluated the effect of above-mentioned stabilizers either alone or in selected blends on rheological and organoleptic properties of AC (Gustaw & Mleko, 2007; Hosseini-Parvar, Matia-Merino, & Golding, 2015; Jana, Patel, Suneeta, & Prajapati, 2010; Kiziloz et al., 2009). Acid CN-based processed cheese analogues (11.5% acid CN in the formulation) obtained with addition of WPI (0.5% in the formulation) and different hydrocolloids blends (up to 0.21% in the formulation) showed increased hardness and decreased adhesiveness (Gustaw & Mleko, 2007). The magnitude of these modifications varied as a function of the amount of hydrocolloids blends. The addition of κ-carrageenan:locust bean gum (ratio 1:1) or xanthan gum:locust bean gum (ratio 1:1) blends in the range 0.07–0.21 caused a linear increase in AC hardness and a decrease in adhesiveness independently of the blend used. These effects were explained by the synergistic interaction between these polysaccharides. Probably the interaction between xanthan gum or carrageenan with galactomannan was stronger than the polysaccharide with CN and ultimately polysaccharides created a separate gel network.

Hosseini-Parvar et al. (2015) explored the impact of basil seed gum (a surface-active polysaccharide-based hydrocolloid) on functional properties of AC. Analogue cheeses (52% moisture, 30% soy oil contents) were manufactured at different levels of proteins (6–10%), water (56–52%) and basil seed gum (0–1%). Increasing levels of added hydrocolloid in the formulations with the same protein content contributed to oil emulsification. By adding basil seed gum, it was possible to manufacture AC with higher firmness, but slightly lower meltability, and at lower cost owing to the lower protein and higher moisture contents. For instance, replacing 4% of the protein content (i.e. from 10% to 6%) with 0.1% basil seed gum and 4% water (from 52% to 56%) allowed to obtain an AC with slightly lower meltability than the sample with 10% protein (0% basil seed gum and 52% moisture contents). The rationale was the formation of a hydrocolloid network capable to strengthen the network formed by casein strands.

Acidifying agents

The combination of several factors guarantees microbial stability of AC during storage. Indeed, AC stability would rapidly decrease at pH levels of the homogenous hot molten blend (~ 8–9) (Guinee, 2007). Thus, the addition of acidifying agents allows the lowering of pH value, to average values of 6.0–6.6 for a generic AC (Fig. 2). Guinee (2011) reported that, as a function of the final end-use application, the final pH value may significantly vary within the same variety. For instance, in the case of MCA the final pH may range from 6.1 to 6.6. Besides the preservation action, the acidification of the molten mass exerts also a flavor contribution to the final product. Acidifying agents include acetic, adipic, citric, lactic, malic and phosphoric (Fox et al., 2017b; Guinee, 2011). The acids most frequently added are citric or lactic acid. Food-grade organic acids are used at concentrations of ~ 0.2–1%, as a function of the type of acid (see also Table 1). Also types of ES, having different pH-buffering capacity, affect subsequently the concentration of acidulants added in the formulation.

Preservatives

An array of compounds are added as preservatives in AC processing., including potassium sorbate, nisin and/or calcium/sodium propionate. Typically, they are used as shelf life extenders to inhibit or retard the growth of microorganisms at levels of 0.1% (Guinee, 2007). In a complex matrix, such as AC, the combined use of preservatives may enhance the antimicrobial activity. Thus, a careful integration of preservatives should be considered because several factors such as antagonistic interactions with food components, pH or storage temperature could affect the microbial stability. Recently, Khanipour et al. (2016), testing different combinations of preservatives (NaCl, potassium sorbate, nisin), developed a logistic regression model enabling prediction of the probability of growth of *Clostridium sporogenes* in a high moisture (60%) ambient shelf-stable processed cheese analogue. This microorganism was selected having similar growth characteristics to the pathogen *C. botulinum*, but not being dangerous. They outlined that NaCl at 3% (a relatively high concentration) alone did not prevent the growth of *C. sporogenes* in the high moisture AC samples (at pH 5.5). Differently, proper combinations of NaCl (up to 3%), potassium sorbate (up to 0.2 %) and nisin (up to 240 ppm) were successful inhibitors. The model enabled the level of probability to be set to provide a required level of stringency.

Miscellaneous food additives

An array of compounds is adopted in AC manufacture such as flavor, flavor enhancers, colors, minerals and vitamin preparations. Flavor is a key sensory attribute affecting consumer acceptance of AC. Generally, an important difference of AC in comparison to the natural counterpart is considered the bland flavor. The use of little amounts of cheese (<50 mg/kg of the final product, Fox et al., 2017a) is a way to overcome this defect and impart flavor. An alternative is the use of dairy cream instead of vegetable oil/fat. However, the latter solution is detrimental in terms of costs. Due to certain limitations in the addition of natural cheeses, the industrially-preferred strategy is the inclusion of flavoring agents. Flavor compounds include enzyme-modified cheeses (EMC), smoke extracts and starter distillates, whose typical addition level ranges from 0.5% to 3.0% (Guinee, 2007). The inclusion in the formulation is preferably carried out towards the end of the processing to minimize the loss of volatiles (Guinee, 2011). Enzyme-modified cheeses are concentrated cheese flavors, which are 5–20-fold more intense than corresponding natural cheese (Fox et al., 2017b). They offer the advantages of high flavor intensity and stability. Composition and flavor components of EMC have been reviewed by Kilcawley, Wilkinson, & Fox (1998). Noronha, Cronin, O'Riordan, & O'Sullivan (2008a, 2008b) reported that the quality of EMC-flavored AC is affected by various parameters, such as the amount of flavor-active hydrolysis substances in the EMC (i.e. free fatty acids), the pH and the composition of AC. In details, these authors analyzed medium (13%) and low (2%) fat AC (pH 6.0 or 5.5) flavored with 5% EMC at 16%, 28% or 47% total free fatty acids (low to high levels of hydrolysis, respectively). Following sensory analysis by ranking tests, the acceptability by panellists of low (2%) fat AC was optimized by adding EMC with medium level (28% total free fatty acids) of lipolysis. 'Stronger' flavors were perceived in AC with pH of 5.5, because of the higher levels of free fatty acids than those formulated with a higher pH. Irrespective of the degree of hydrolysis of the EMC used, AC with 13% fat showed higher flavor release properties than those containing 2% fat. Low-fat cheeses flavored (pH 6.0 or 5.5) with 28% lipolysis EMC were described as 'well-balanced' and 'cheesy' and were preferred over AC containing high hydrolysis EMC.

Sodium chloride contribute to the flavor of AC and its content may be as high as 2–3% (El-Bakry, Beninati, Duggan, O'Riordan, & O'Sullivan, 2011d). The contribution of NaCl to overall sodium content of AC is ~ 60% of the total sodium (the remaining ~ 40% is due to ES). On the basis of these data, recently the interest of research addressed to the reduction of NaCl in AC (Table 2). Gustaw and Mleko (2007) investigated the influence of NaCl concentration (from 0.5 to 2% in the formulation) on texture properties of AC formulated with 11.5% acid CN, 0.5% WPI and 0.07–0.21 hydrocolloids blends (see also the section Hydrocolloids). Independently to the hydrocolloid blend used, increasing the NaCl concentration boosted linearly the effect of the increased concentration in hydrocolloids, i.e increased AC hardness and decreased adhesiveness. This phenomenon was explained by the influence of cations (sodium of NaCl) on the gelling behavior of polysaccharides. In a further work on this topic, El-Bakry et al. (2011d) studied the effects of different NaCl concentrations (0−1.5%) in rennet CN-based AC (48% moisture) on physico-chemical properties, cheese manufacture and functionality of the final product. On a small-scale cooker (Farinograph, 800 g of AC/batch), the authors observed that reducing NaCl from 1.5% up to 0% resulted in a progressive reduction in total mixing energy and total processing time, an increase in pH value (from 6.0 up to 6.5), an increase in water activity (from 0.975 up to 0.982), an increase in fat globule diameter (from 18 up to 29 µm), a significant decrease in hardness and a slight increase in flowability on melting. A similar trend of results was obtained using a pilot-scale cooker (Blentech, 4 Kg/batch). The higher pH value observed upon the reduction of NaCl was ascribed to the less displacement of hydrogen from the CN by sodium. This result reflected in higher net negative charges of CN molecules and higher electrostatic repulsions between them, leading to an increase in protein hydration. Shorter processing times, i.e. less shearing of the molten mass led to bigger fat globules. Both the increase of fat globule size and the increase in pH contributed to changes in post-manufacture cheese properties (hardness and flowability). Microbial count was 10^3 cfu g^{-1} after manufacture reaching ~ 10^4 cfu g^{-1} after 4 weeks (at 4 °C) independently of NaCl content. Anyway, at 0% NaCl microbial stability was reduced after 12 weeks (10^6 cfu g^{-1} while in AC with 1.5% NaCl remained at ~ 10^4 cfu g^{-1}). From time 0 to 4 weeks storage moulds and yeasts were absent in control AC (with 1.5% NaCl). Differently, the yeasts count after 2 weeks reached 10^3 cfu g^{-1}. Both pH and water activity increases were responsible of the reduced microbial stability. On the whole, the authors remarked the feasibility to reduce NaCl content in AC by adjusting of the final pH value with citric acid.

Flavor enhancers are facultatively adopted in AC formulations. This group of compounds includes glutamates and yeast autolysates (Chandan & Kapoor, 2011). A steadily revised list of permitted food additives including the maximum use levels in AC was provided by the Commission of Codex

Alimentarius (2016). The functional classes reported in the list include acidity regulators, colors, emulsifying agents, flavor enhancers, preservatives and stabilizers.

Colors included in AC formulation (annatto, paprika, artificial colors) are optional ingredients and typically are added at concentrations of 0.04% on average (Guinee, 2007). Mineral and vitamin preparations introduced as nutritional supplements (at mean rates of 0–0.5%) (Chandan & Kapoor, 2011), include magnesium oxide, zinc oxide, iron, vitamin A, palmitate, riboflavin, thiamine and folic acid (Guinee, 2011).

Processing conditions

The manufacture of AC is similar to that of processed cheeses and relatively simpler in comparison to that of natural ones. The main steps of AC processing consist of formulation, mixing, heating and shearing, hot packaging and cooling. The entire process is accomplished within in 8–13 min. Most protocols are patented, but a general description of each step has been reported in literature (Fox et al., 2017a). For the sake of simplicity, we provide an example of processing flow-chart of AC in Fig. 2. The sequence of addition of ingredients may vary as a function of plant practices, plant design, type of ingredients, processing parameters and required performances. In general, dry ingredients (casein, caseinate, whey protein derivatives, ES, starch, preservative) and water are dosed in the processing kettle, kept under agitation, heated by direct steam injection to ~ 50 °C and blended for 2–3 min (Fox et al., 2017a). Schatz et al. (2014) recommended a procedure to pre-disperse the CN and hydrate dry ingredients in hot solution, followed by addition of ES. This procedure allows obtaining AC with proper consistency, good flow of the melt and no impairment of melting properties. When rennet CN is the protein source, a high pH (> 7) is preferred, because it contributes to a greater sequestration of calcium from CN by the action of ES, enhancing the negative charge of CN. Under these conditions, the calcium phosphate *para*-CN is converted to sodium *para*-caseinate. The latter, reducing the free interfacial energy of the fat phase, emulsifies the dispersed oil droplets during processing, thus contributing to the formation of a stable oil-in-water emulsion. After fat addition (typically, soy oil, palm oil, rapeseed oil and/or their hydrogenated corresponding fats) the mix is heated to ~ 85 °C and kept under agitation for 5–8 min until a hot molten mass is formed. After addition of acidifying agents and flavors under agitation (see also sections Acidifying agents and Miscellaneous food additives), it follows setting and structure formation during cooling. Cooking conditions (time and temperatures) have an influence on the functional properties of the final product (Shabani et al., 2016). Typically, an increase in cooking temperature and time as well as in shearing intensity promote an increase in hardness and a concomitant decrease in spreadability and meltability of AC spreads (Mulsow et al., 2007). Shear rate affects the degree of emulsification of fat in the cheese-like matrix. Different types of in-batch cookers with specific design and operating conditions are available for AC processing. Such plants are generally adopted in the manufacture of batches of 2–4 kg. The most common type has single/twin-screw augers with agitation speeds ranging from 100 to 200 rpm (Blentech cooker). This reduced shear action promotes a low fat dispersion, resulting in large fat particles (diameter 5–25 µm). Consequently, the final AC upon baking is characterized by the desired performances when used as pizza topping (i.e. sufficient degree of oiling off and limited dehydration) (Fox et al., 2017a). An alternative to the twin-screw cooker is the single-blade cooker (Stephan cooker, generally adopted for processed cheeses), which is characterized by higher speeds (up to 1,500 rpm). Noronha, O'Riordan, and O'Sullivan (2008d) compared the performances of cheese system in cheese plants and focused on the effect of different agitation speeds (100 to 200 rpm with Blentech and 750 to 1,500 rpm with Stephan) at a constant holding time (~ 2 min) at 80 °C. The authors observed that increasing the speed of agitation in the Blentech cooker slightly affected hardness and meltability values of AC, whereas increasing the blade speed of the Stephan cooker caused a decrease in the size of fat globules with a significant increase in the hardness and a decrease of the meltability of AC. They concluded that functional properties (texture, melting and color) of the end-product were acceptable also in single-blade cooker when operating at moderate speeds (750 rpm).

An additional manufacturing step dedicated only to AC spreads or dips is homogenization (Guinee, 2011). Essentially, this phase promotes further mixing, shearing and interactions of blend ingredients. Only at the end of manufacture, flavors as well as food-grade acids are added at concentrations above mentioned. At this stage of processing, additional ingredients can be introduced, namely: hydrocolloids, colors, preservatives, minerals and vitamin preparations (Guinee, 2011). Hot packaging and refrigerated storage follows after 1–2 min of blending. Through cooling, the homogeneous the and viscous mass sets to form a structure, variable from soft and spreadable to firm and sliceable matrix is converted to a variable from soft and spreadable to firm and sliceable. Molding and hot packing are followed by the cooling step (Fig. 2). The subsequent storage conditions of AC are important because can affect physical properties and final texture (Mulsow et al., 2007).

Functional properties of analogue cheeses *vs* natural cheeses and changes upon storage

In the US, the principal application of AC is as topping in Pizza pie where low-moisture MCA is the main type used (Guinee, 2011). The concept that cheese functionality is the major contributor to its specific use is well exemplified by the consumption of MC/MCA as ingredients in Pizza pie. Typically, upon baking the prevailing expected attributes are high stretchability, together with moderate flowability, oiling-off and chewiness to form strands of molten cheese when slices of Pizza are separated (McMahon & Oberg, 2011). A survey of commercial samples of MC (n = 8) and MCA (n = 8) of Danish, Irish and UK origin revealed inter- and intra-variety differences in composition, microstructure and functionalities upon baking (Guinee, Harrington, Corcoran, Mulholland, & Mullins, 2000). Samples of MCA exhibited gross composition parameters slightly different to those of MC, the former having meanly higher amounts of moisture (48.8% *vs* 46.4%), fat (25.0% *vs* 23.2%) and lower levels of protein (18.4% *vs* 26.0%). This compositional variability was justified also by the use of polysaccharides in MCA formulation, as protein replacer to reduce costs. Observed microstructural differences between the two varieties reflected the different distributions of para-CN and fat phases. In MC para-CN consisted of aligned protein fibers with entrapped fat columns made of coalesced and discrete globules. More uniformly distributed para-CN matrix including similarly-sized fat globules were observed in MCA (Guinee et al., 2000). Functional parameters varied significantly between MC and MCA. In particular, the mean value of stretchability for the melted MC was significantly higher, while the differences in terms of flowability were not significant (due to the high intra-variability). Also the values of the mean apparent viscosity, which is a measure of the chewiness of the melted cheese, were similar. On the whole, differences in functionalities of the baked MC and MCA samples reflected differences in processing, composition, stage of ripening/storage and/or formulation in the case of MCA (Kindstedt, 1993; Renda, Barbano, Yun, Kindstedt, & Mulvaney, 1997).

The properties of MC used as Pizza topping are dynamic, undergoing to age-related changes (Fox et al., 2017b; Kindstedt, 1993). A major role in these modifications is played by the extent and depth of proteolysis, which in turn is influenced by parameters such as the curd manufacturing process, conditions of plasticization, type and residual activity of coagulant, type of starter and storage temperature. Feeney, Fox, and Guinee (2001) reported that the increase in the storage temperature of MC from 0 °C to 15 °C (up to 70 d) reflected in an increased extent of proteolysis, without altering the type of proteolysis. In details, the decrease in intact CN with the increase of temperature was consistent with α_{s1}-CN degradation. The same authors (Guinee, Feeney, & Fox, 2001) recorded that the decrease of intact CN during ripening (up to 70 days) at 0, 4, 10 or 15 °C was always paralleled by a linear decrease in firmness. Under these conditions, also functionalities of the baked MC changed upon storage. During the first 30 d of ripening, melt time and chewiness decreased, whilst flowability and stretchability increased and maintained this status up to 60 days. These improvements were promoted by the decrease of intact para-CN and the increase in protein hydration (swelling of para-CN fibers). In comparison to MC, the absence of a ripening period makes MCA a less dynamic system in which physicochemical and microstructural changes are very limited within the typical commercial storage interval (1 month). This behavior was observed by Marshall and Kirby (1988) who reported no texture changes in AC during the first 10 days of storage. Kiely, McConnell, and Kindstedt (1991) observed that flowability, stretchability and apparent viscosity of MCA were stable during storage at 4 °C even up to 150 days.

Also microbiological properties of AC differ from those of the natural cheeses. In most natural cheeses, selected strains of lactic acid bacteria (LAB) are added to the milk. In a freshly made cheese these bacteria rapidly reach levels of ~ 10^9 cfu g^{-1} and during ripening decrease. For instance, in Cheddar the LAB count within about 10 days passes from ~ 10^9 cfu g^{-1} to ~ 10^5 cfu g^{-1}. Meanwhile, the non-starter

LAB increase from ~ 100 cfu g^{-1} to ~ 10^{5} cfu g^{-1}, reaching ~ 10^{7} cfu g^{-1} after 1 month ripening (Cogan, 2011). This complex microbial ecosystem contributes to the biochemistry of ripening modifying cheese texture and functionality. Differently, in AC processing no starter bacteria are added, thus microbial counts are attributed only to environmental contamination. Laboratory made AC with total viable counts of 10^{3}−10^{4} ufc g^{-1} were considered normal and acceptable in AC freshly made or after 1 week at 4 °C (Dhanraj, Jana, Modha, & Aparnathi, 2017; O'Malley, Mulvihill, & Singh, 2000), because the manufacturing environment is not sterile. Throughout storage at 8 °C of commercial samples of rennet CN-based MCA, O'Malley et al (2000) observed an increase of microbial counts, which plateaued 10^{5}−10^{6} ufc g^{-1} between 4 and 8 weeks and increased up to 10^{7}−10^{8} ufc g^{-1} after 32 weeks of storage. In this case, likely both the high pH value (6.5) and the storage temperature (8 °C) contributed to the microbial growth.

Conclusions

Over the years, the image of AC changed from products made with ingredients of low quality to functionalized ingredients, fulfilling desirable nutritional-dietary properties (cholesterol free, reduced saturated fats, reduced sodium). Besides the development of products meeting consumers' expectations also cost-effectiveness represents a key driver explaining the positive outlook for AC market. The exemplary potential of AC production is also consistent with the upside trend of food service and food industry. In this context, scientific research yielded insights both on process optimization for AC protocols to best fit specific applications and on parameters/conditions making feasible AC customization. Outcomes evidenced how proper selection of ingredients and fine-tuning of formulation putatively offer the chance to manufacture a wide array of AC. Major topics of interest focused on effects of ingredient selection and processing conditions over nutritional and functional properties (texture, rheological and melting) of AC. A challenge for future studies is to pursue a deeper knowledge on the action mechanism and the synergy of ingredients on AC structure and functionalities. This upgrade could improve the feasibility of industrial scale-up manufacture of AC. The optimization of sensory properties, being crucial for sales and marketing, represents the leitmotif for today’s manufacturers, as a way, for instance, to improve the consumers’ perception of low-fat AC. The use of new added-value ingredients could entail the development of new versatile and cost-reduced varieties.

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Figure Captions

Fig. 1 - Factors affecting the rheology of analogue cheeses (AC).

Fig. 2 - Generalized manufacturing protocol of an analogue cheese.

Table 1 – Examples of AC formulations reported in literature (amounts expressed as percentage, w/w)

	Type of AC					
Ingredient	MCA[A]	MCA[B]	Soft/tough[C]	Processed[D]	Spread[E]	Spread[F]
Acid CN	21.0	-	-	11.5	-	-
Rennet CN	-	20.0−22.3	21.7	-	-	-
Sodium caseinate	-	-	-	-	20.9−24.5	-
Calcium caseinate	-	-	-	-	-	11.9
WPC	-	1.2−3.5	-	-	0−3.0	-
WPI	-	-	-	0.5	-	-
Vegetable oils/fats	12.5	15.0	11.1−21.0	30[a]	14.0	23.0
Emulsifying salts	2.8	2.5	1.3	2.0	0.5	0.8
Tween 80	0.2	-	-	-	-	-
Starch	5.0	-	0−9.9	-	-	-
Gums	0.4	-	-	0.07−0.21	0−0.6	-
Sodium chloride	1.1	1.1	1.4	0.5−2.0	1.3	1.3
Preservatives	-	-	0.1	-	0.2	-
Flavors	0.3	1.5	-	-	-	-
Acidifying agents	0.3	0.6	0.5	to pH 6.2	1.0	-
Calcium chloride	0.4	0.1	-	-	-	-
Water [b]	56.4	55.7	54.0	to 100%	52.0	63.1

[a]: anhydrous milk fat; [b]: inclusive of condensate; AC: Analogue cheese; MCA: Mozzarella cheese analogue; CN: casein; WPC: whey protein concentrate (68% protein in Ref. [B]; 35% protein in Reference [E]); WPI: whey protein isolate (92% protein).

References. [A]: Jana & Upadhyay, 2001; [B]: Dhanraj et al., 2017; [C]: Noronha et al., 2008c; [D]: Gustaw & Mleko, 2007; [E]: Hosseini et al., 2014; [F]: Cunha et al., 2013.

Table 2 – Recent research topics in analogue cheese formulations and manufacture

Topic of investigation	References
Starch as R-CN replacer	Mounsey & O'Riordan, 2008a, 2008b, 2008c, 2008d; Trivedi et al., 2008; Ye & Hewitt, 2009; Ye, Hewitt & Taylor, 2009
WPC as partial R-CN replacer in MCA	Dhanraj, Jana, Modha, & Aparnathi, 2017
WPC and guar gum in sodium caseinate-based AC	Hosseini, Habibi Najafi, & Mohebbi, 2014
Starch and hydrocolloids as R-CN replacers	Kiziloz, Cumhur, & Kilic, 2009
Starch as fat replacer	Duggan, Noronha, O'Riordan, & O'Sullivan, 2008; Mounsey, 2009; Noronha, Duggan, Ziegler, O'Riordan, & O'Sullivan, 2008c
Types of hydrocolloids and fats	Hanáková, Buňka, Pavlínek, Hudečkhová, & Janiš, 2013
Fat replacement with inulin	Hennelly, Dunne, O'Sullivan, & O'Riordan, 2006
Types of ES	El-Bakry, Duggan, O'Riordan, & O'Sullivan, 2011b
Potassium- based *vs* sodium-based ES	El-Bakry, Duggan, O'Riordan, & O'Sullivan, 2011a; Schatz, Hoffmann, Schrader, & Maurer, 2014
ES-free AC to reduce sodium and for specific end-use applications	McIntyre, O'Sullivan, & O'Riordan, 2017
NaCl reduction	El-Bakry, Beninati, Duggan, O'Riordan, & O'Sullivan, 2011d
Types of preservatives and microbial safety	Khanipour et al., 2016
Microwave expanded AC	Arimi, Duggan, O'Sullivan, Lyng, & O'Riordan, 2010, 2011, 2012
Standardization of MCA manufacture	Shah, Jana, Aparnathi, & Prajapati, 2010
Scale-up manufacture	El-Bakry, Beninati, Duggan, O'Riordan, & O'Sullivan, 2011d

AC, analogue cheese; A-CN, acid casein; ES, emulsifying salts; MCA: Mozzarella cheese analogue; R-CN, rennet casein; WPC, whey protein concentrate.

Fig. 1

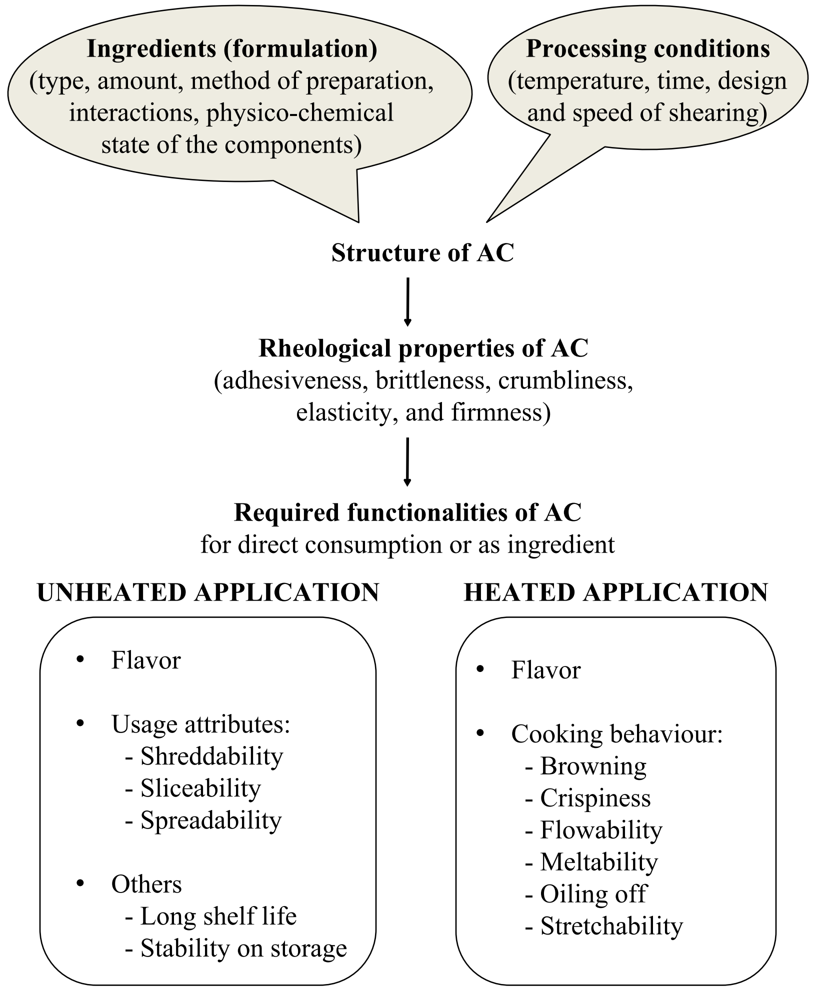
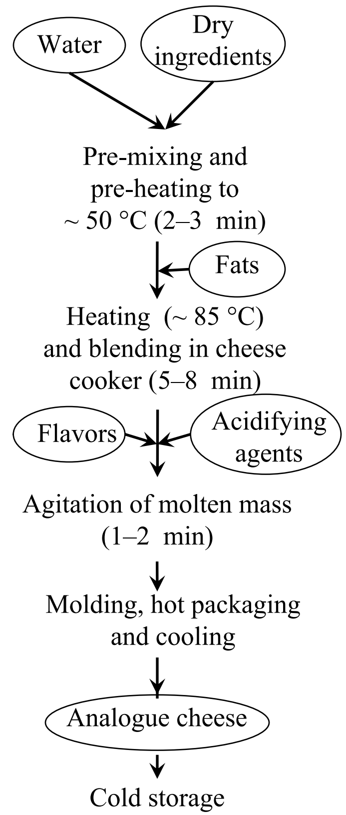

Ingredients (formulation)
(type, amount, method of preparation, interactions, physico-chemical state of the components)
Processing conditions
(temperature, time, design and speed of shearing)
Structure of AC
Rheological properties of AC
(adhesiveness, brittleness, crumbliness, elasticity, and firmness)
Required functionalities of AC
for direct consumption or as ingredient
UNHEATED APPLICATION
• Flavor
• Usage attributes:
- Shreddability
- Sliceability
- Spreadability
• Others
- Long shelf life
- Stability on storage
HEATED APPLICATION
• Flavor
• Cooking behaviour:
- Browning
- Crispiness
- Flowability
- Meltability
- Oiling off
- Stretchability

Fig. 2


Water
Dry ingredients
Pre-mixing and pre-heating to ~ 50 °C (2–3 min)
Fats
Heating (~ 85 °C) and blending in cheese cooker (5–8 min)
Flavors
Acidifying agents
Agitation of molten mass (1–2 min)
Molding, hot packaging and cooling
Analogue cheese
Cold storage