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Investigating The Bioactive Properties of Cheese-Fruit Combinations Following *in Vitro* Digestion Using an Elderly Model

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Abstract

The prevalence of disease in older adults is increasing, thus there is a need to develop functional foods for this cohort that can promote healthy aging. This study analyzed cheese combined with fruit to identify if certain cheese-fruit combinations improved the bioactive properties of the cheese. Feta, Reduced-Fat Red Cheddar (RFRC), and Goat's cheese were combined with different fruit (goji berries, red pepper, or blackberries) and digested with a simulated gastrointestinal *in vitro* digestion model representative of older adults. Antioxidant potential was investigated using DPPH radical scavenging, Ferric reducing antioxidant power (FRAP) and Total phenolic content (TPC) assays. The ability of samples to inhibit digestive enzymes was determined using the α-glucosidase inhibition assay. Antimicrobial activity against *Listeria monocytogenes,* Group B *Streptococcus and Escherichia coli* was investigated by the disc diffusion method. Immunomodulatory potential of the digestates was evaluated by their ability to modulate TNF-α levels in stimulated Jurkat T cells. Results demonstrated that combining RFRC with all fruit significantly ($p<0.05$) increased both the antioxidant and α-glucosidase inhibitory potential of the cheese (≥90.6% DPPH inhibition, ≥980.5 FRAP μmol Fe^{2+}/kg.fw, and ≥58.1% α-glucosidase inhibition). Reducing potential of all cheese significantly ($p<0.05$) increased when combined with fruit (≥977.0 FRAP μmol $Fe^{2+}/kg._{fw}$). Group B *Streptococcus* was inhibited by cheese-fruit combinations containing feta and goat's cheese. Combining fruit with feta altered the immunomodulatory potential of the cheese by significantly ($p<0.05$) decreasing TNF-α secretion by ≥41%, compared to the control. Novel cheese-fruit combinations that promote synergistic bioactive properties could help design functional foods for older adults that promote healthy aging.

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Introduction

As people are living longer,[1] the prevalence of chronic conditions, including diabetes mellitus, cancer and cardiovascular disease is increasing.[2] As we age, our immune system alters and older adults, particularly those who are immunocompromised, are more susceptible to infection.[3] Evidence suggests that good nutrition plays a key role in promoting healthy aging.[4] It has been suggested that incorporating the right foods and nutrients in the diet can help reduce the incidence of age-related diseases,[5] and the need for pharmaceutical interventions.[6,7] Furthermore, incorporation of whole foods may be more beneficial than food supplements, as often isolated compounds lose bioactivity.[8]

Fruit and dairy are essential dietary components.[9,10] Fruits provide vitamins and minerals, and are also a rich source of phytochemicals that act as antioxidants,[11] which can help prevent chronic illnesses and reduce disease-related mortality.[12] Dairy products (milk, yogurt and cheese) are a good source of calcium,[13] which helps prevent bone loss in the elderly,[14] and are also a source of bioactive compounds that have many associated health benefits.[15,16] Individually, cheese and fruit consumption have been associated with a reduced risk of type 2 diabetes.[17,18] The antimicrobial potential of cheese has also been well documented[19–23] and few studies have reported on the antimicrobial properties of fruit including berries, blackcurrants, grapes and peppers.[24–26] Despite the potential health benefits, the Irish Longitudinal Study on Aging reported that 75% of older adults do not consume the recommended dietary allowance (RDA) of fruit and vegetables, and 70% of the older adult population consume less than the recommend daily serving of dairy.[27]

Combining fruit and dairy products has the potential to promote synergistic health benefits.[28] Food synergy relates to combined food ingredients or food matrices that demonstrate improved health benefits beyond their basic nutritional composition, compared to the individual foods or ingredients.[29] Combining foods or food ingredients can also have antagonistic effects,[30] whereby the sum of the effects is less than that from the individual components.[31] Previous studies have demonstrated that fortifying dairy products with herbs and fruit, has the potential to improve the antioxidant, antidiabetic and antimicrobial properties of the dairy food. Al-Otaibi *et al*.,[32] reported improved antioxidant properties for mold-ripened cheese following fortification with date palm fruit, while Apostolidis *et al.*,[33] reported the ability of cranberry enriched cheese to inhibit key enzymes relevant to carbohydrate metabolism. In addition, Khalifa and Wahdan.,[34] demonstrated that the addition of cranberry fruit extract to soft white Domiati cheese significantly improved the antimicrobial properties of the cheese and reduced microbial growth during storage.

Studies have highlighted the potential health benefits of combining dairy and fruit, however; to the best of our knowledge, the bioactive potential of cheese-fruit combinations following *in vitro* digestion has not been reported. Rashidinejad *et al*.,[35] investigated the effects of combining a full-fat hard cheese matrix with green tea catechins on the bioactive properties of cheese, following *in vitro* digestion, and confirmed that the addition of the green tea extracts increased the antioxidant properties of the cheese.

Novel cheese-fruit products could help address the low dietary intake of dairy and fruit in the older adult population.[27] In this study, selected cheese matrices were combined with different fruit and then digested using a simulated gastrointestinal *in vitro* digestion (SGID) model representative of older adults. The digestates were then assessed to determine if the combinations altered the potential antioxidant, α-glucosidase inhibitory and immunomodulatory properties of the cheese. The antimicrobial properties of the cheese-fruit combination were also investigated. Cheese-fruit combinations with demonstrated synergistic effects could be considered as functional foods for older adults with potential to promote healthy aging.

Materials and Methods

All chemicals were purchased from Merck (Sigma-Aldrich, Ireland), unless otherwise stated. The Jurkat cell line was purchased from the European Collection of Authenticated Cell Cultures (ECACC, UK). Bacterial cultures included *Escherichia coli* (#DSM3008; DSMZ, Germany), *Listeria monocytogenes*[36] and Group B *Streptococcus* (GBS).[37] Antimicrobial agents included Gentamicin

(10 µg) and Penicillin G (10U) (Oxoid, Thermofisher scientific, Ireland). Ringers tablets, brain heart infusion (BHI) broth and BHI agar were purchased from LAB M, UK.

Sample Selection and Preparation

Cheese and fruit products were purchased from local supermarkets (within one season) and included reduced fat red cheddar (RFRC), feta, goat's cheese, goji berries, red peppers, and blackberries. Cheese products were selected to represent cheese with different textures and fat content, as it has previously been reported that cheese varying in texture and fat content have different digestibility rates.[38] The red cheddar was a reduced fat, hard cheese made from cows' milk; the feta was a full-fat soft cheese made from sheep and goat's milk, and the goat's cheese was a medium-fat soft cheese made from goat's milk. Three types of fruit including goji berries, red pepper and blackberries were selected as they are all known to be good sources of antioxidant compounds and are associated with a range of bioactive properties.[39–42] Food combinations were prepared by mixing 18 g of cheese with 2 g of fruit in 20 mL of distilled deionized H_2O for 2 min. The composition of each cheese, as identified by the manufacturer, is summarized in Table 1.

Table 1: Composition of cheese products purchased from local supermarkets

Cheese type per 100g	Energy (Kcal)	Fat (g)	Saturates (g)	Protein (g)	Salt (g)
Reduced fat red cheddar	302.0	22.0	14.0	28.0	2.0
Feta	279.0	23.0	17.1	16.9	1.9
Goat's cheese	158.0	12.0	8.0	9.5	1.3

***In vitro* Digestion Procedure**

To investigate and compare the potential bioactive properties of the cheese-fruit combinations, each combination (10 % w/w) was subjected to simulated gastrointestinal *in vitro* digestion (SGID) using a method described by Plante *et al.*,[43] modified from the SGID model described by Minekus *et al.*,[44] to reflect some of the physiological changes associated with an older adult. Briefly, 5 g of sample was homogenized with 3.5 mL of simulated salivary fluid, 25 µL $CaCl_2$ (0.3M) and water to a final volume of 10 mL, and then incubated at 37°C in a shaking incubator for 2 min. Following this, the gastric phase was simulated by mixing 10 mL of oral digestate with 7.5 mL simulated gastric fluid (pH 3), 750 U mL^{-1} porcine pepsin, 5 µL $CaCl_2$ (0.3M), 0.2 mL HCL (1M) and water to achieve a final volume of 20 mL, and incubated at 37°C in a shaking incubator for 2 hr. The final phase of digestion, the intestinal phase, was simulated by mixing 20 mL of gastric digestate with 11 mL of simulated intestinal fluid (pH 6.5), 5.0 mL of pancreatin solution (800 U mL^{-1}), 2.0 mL (10mM) bile, 40 µL of $CaCl_2$ (0.3M), 0.15 mL NaOH (1M) and water to achieve a final volume of 40 mL, and incubated at 37°C in a shaking incubator for 2 hr.

Antioxidant Activity

Chemical-based *in vitro* antioxidant assays are useful screening tools as they are low cost, allow for high-throughput, and yield an index value that helps to compare the potential antioxidant properties of different compounds and products.[45] The principles of the assays can vary and, therefore, it is recommended that more than one assay is used to assess antioxidant activities.[45]

DPPH (2,2-di-phenyl-1-picryl hydrazyl) Radical Scavenging Activity

Cheese digestates were investigated for their ability to scavenge the DPPH free radical using a previously described method,[46] with modifications[43];1mL of sample was mixed with 3 mL of 60 µM DPPH/ methanol solution. Color blanks were prepared with 1 mL of digestate and 3 mL of methanol, and controls consisted of 1 mL of methanol to 3 mL of 60 µM DPPH/methanol solution. % DPPH inhibition was calculated against the control and compared to a Trolox standard curve (0.04 - 0.4 µM).

$$\%\ DPPH\ Inhibition: \frac{[\text{Abs Control} - (\text{Abs Extract} - \text{Abs Colour Blank})]}{\text{Abs Control}} x\ 100\%$$

Ferric Reducing Antioxidant Power

Reducing power of cheese digestates was assessed according to Benzie and Strain.,[47] with modifications[43]; Samples (1 mL) were combined with 2 mL of FRAP reagent and, color blanks were prepared with H_2O in place of FRAP reagent. A mix of FRAP reagent (2 mL) and distilled deionized H_2O (1 mL) was used as a blank. Results were expressed as micromole of ferrous per $kg_{.fw}$ of cheese sample (μmol $Fe^{2+}/kg_{.fw}$).

Total Phenolic content

TPC of cheese digestates was measured by Folin-Ciocalteu method,[48] with modifications.[43] Samples (50 μL) were added to Folin-Ciocalteau solution (250 μL) and incubated for 4 min. Then, 500 μL of 2 % (w/v) Na_2CO_3 and 4.2 mL of H_2O were added. Color blanks consisted of 50 μL of sample, 4.45 mL of H_2O and 500 μL of 2 % (w/v) Na_2CO_3. After 120 min at 20°C, absorbance was measured at 765 nm versus a water blank. TPC was determined against a standard curve of gallic acid (0 – 50 mg/mL) and expressed as mg gallic acid equivalents (GAE) per 100 g of fresh sample (mg GAE/100g.fw).

α-glucosidase Inhibition

The ability of digestates to inhibit α-glucosidase, an enzyme responsible for the digestion of complex carbohydrates *in vivo*,[49] was investigated using a previously described method.[33] Sample (50 μL) was mixed with 0.1 M phosphate buffer (100 μL, pH 6.9) containing α-glucosidase solution (1.0 U/mL) in a 96-well plate at 25°C for 10 min. Then, 50 μL of substrate (5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer, pH 6.9) was added to each well. Color blanks consisted of 50 μL of 0.1 M phosphate buffer (pH 6.9) in place of enzyme, and the blank was a mixture of buffer and substrate. Reactions were incubated at 25°C for 5 min. Absorbance was recorded at 405 nm by a microplate reader (Varioskan Flash microplate reader, Thermo Scientific). Results were compared to the control which had 50 μL of buffer solution in place of the extract. The α-glucosidase inhibitory potential was expressed as % inhibition and calculated as follows:

$$\%\ Inhibition: \frac{[\text{Abs Control} - (\text{Abs Extract} - \text{Abs Colour Blank})]}{\text{Abs Control}} x\ 100\%$$

Immunomodulatory Activity

Cytotoxicity was investigated using the MTT assay previously described by Gabrani *et al.*,[50] with modifications. Digestates were analyzed at concentrations of 0-5 % (v/v). Controls consisted of no digestate and media only used as a blank. Cell viability was calculated as follows.

$$\%\ Cell\ viability: \frac{(\text{Abs Sample} - \text{Abs Blank})}{\text{Abs Control}} x\ 100\%$$

Non-toxic concentration of 0.5 % (v/v) was selected to analyze the immunomodulatory properties, with >80 % average cell viability. Immunomodulatory potential was investigated using a previously described method with modifications.[51] Digestates were examined for their potential to modulate TNF-α levels in stimulated Jurkat T cells that were grown in T25 culture flasks (2 x 10^5 cells per mL) with reduced serum media (RPMI/FBS 5 %). Cells were treated with concanavalin A (conA, 50 μg/mL), and incubated in a 96 well plate (100 μL per well) with 0.5 % (v/v) sample at 37˚C in a 5 % CO_2 atmosphere for 24 h. Controls included; (i) Media (5 % RPMI/FBS), (ii) Cells and media (5 % RPMI/FBS), and (iii) Cells treated with conA and media (5 % RPMI/FBS). Plates were then centrifuged (106 g x 10 min), and supernatants collected and stored at -80ºC until analyzed. TNF-alpha was measured by ELISA (Human TNF-alpha, R & D systems), and absorbance was read at 450 nm on a microplate reader (Varioskan Flash microplate reader, Thermo Scientific). TNF-alpha production was determined using online analysis software (elisaanalysis.com) and expressed as pg/mL.

Antimicrobial Activity

Antimicrobial activity of digestates was screened using a disk diffusion assay according to a method described by Meira *et al*.[52] Bacterial cultures of *Escherichia coli, Listeria monocytogenes* and Group B *Streptococcus* were diluted in Ringers buffer to prepare suspensions at 10^8 cfu/mL. Cultures were inoculated onto BHI agar plates using a sterile swab. Sterile discs were placed aseptically onto the surface of the plates, 15 μL of digestate was added to the discs and incubated at 37°C for 24 h to identify zones of inhibition. Antibiotic discs used as positive controls included Penicillin G (10 U) (for GBS and *L. monocytogenes* cultures) and Gentamicin (10 μg) (for *E. coli* cultures). Negative controls consisted of sterile H_2O. Interpretation of growth inhibition was based on measurement (mm) of zones of clearing.

Statistical Analysis

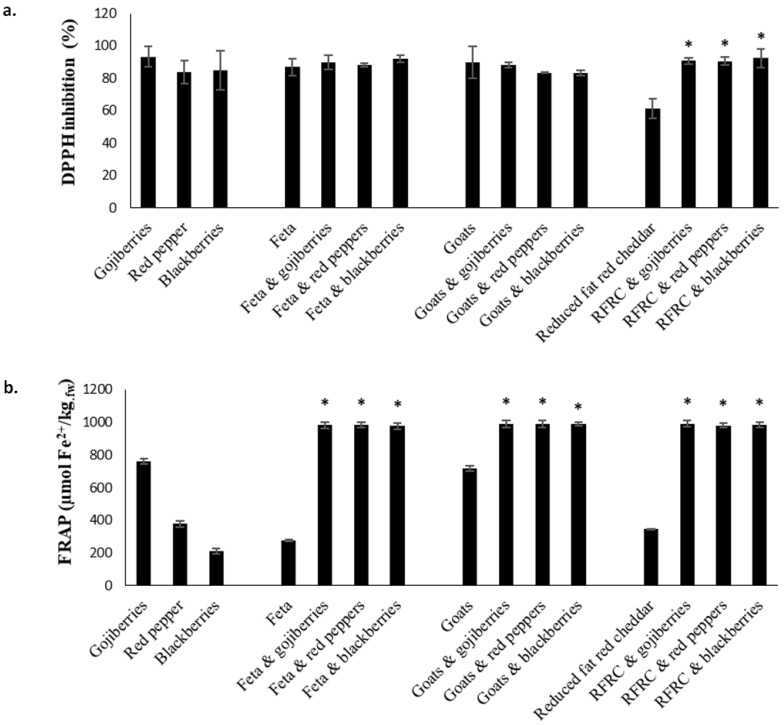
All data was summarized with a mean ± standard deviation of at least three independent experiments. Statistical analysis was carried out using the IBM Statistical Package for Social Sciences (SPSS v.26). All the data satisfied the conditions of normality and homogeneity of variance, hence a one-way analysis of variance (ANOVA) was used to compare differences in the bioactivity between samples obtained following *in vitro* digestion. Controlling for multiple comparisons, the Dunnett's post-hoc test was used to evaluate mean changes between each combination group and the control group. All statistical test results were interpreted using a 5% level of significance.

Results

Antioxidant Activity

Antioxidant potential of individual fruits (goji berries, red pepper, blackberries), plain cheese (feta, goats, RFRC) and, cheese-fruit combinations were investigated by measuring radical scavenging properties and reducing potential of the digested food products. Radical scavenging properties of individual fruits and plain soft cheese (feta or goats) were high (≥83% DPPH inhibition, Figure 1a), and this was not further enhanced when the cheese and fruit were combined ($p > 0.05$, Figure 1a, Table 2). However, the radical scavenging properties of hard cheese (RFRC) significantly increased, when combined with all three fruits ($p < 0.05$, Figure 1a, Table 2).

Reducing power of individual fruits significantly differed ($p < 0.05$) from each other with goji berries demonstrating greatest FRAP (762.4 ± 15.2 μmol $Fe^{2+}/kg_{.fw}$, Figure 1b). Goat's cheese had significantly higher ($p < 0.05$) reducing potential (718.0 ± 13.7 μmol $Fe^{2+}/kg_{.fw}$) compared to the feta and RFRC (278.9 ± 4.9, 345.8 ± 2.7 μmol $Fe^{2+}/kg_{.fw}$, respectively, Figure 1b). However, FRAP of all cheese significantly increased ($p < 0.05$) when combined with the fruits, compared to the plain cheese, and also compared to the fruit alone (Figure 1b, Table 2). TPC of individual fruits was significantly different ($p < 0.05$), with goji berries demonstrating greatest levels (710.6 mg GAE/$100g_{.fw}$, Figure 1c). Regarding plain cheese, RFRC had significantly higher TPC levels ($p < 0.05$, 656.4 mg GAE/100g.fw) compared to feta and goat's cheese (379.5, 312.4 mg GAE/$100g_{.fw}$, respectively, Figure 1c). The majority of cheese samples (89%) had significantly ($p < 0.05$) lower TPC levels following the addition of fruit (Figure 1c).



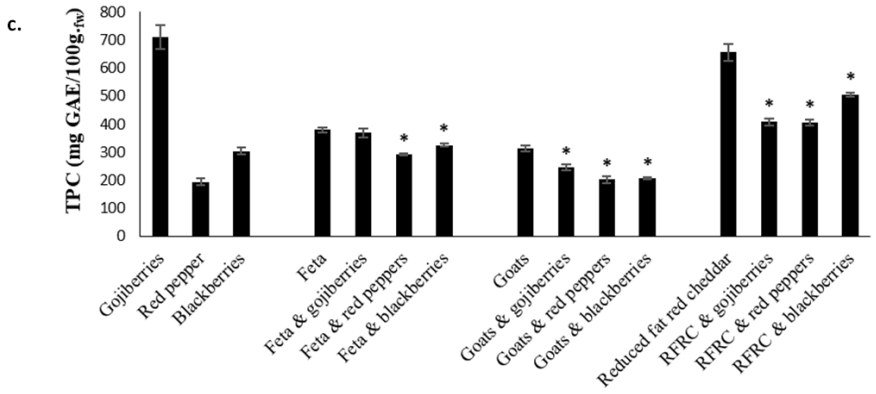


Fig.1: Antioxidant activity of fruit, plain cheese and cheese-fruit combinations following *in vitro* digestion. a. DPPH Inhibition (%), b. Ferric reducing antioxidant power (FRAP), and c. Total phenolic content (TPC). Data represents the mean ± standard deviation of at least three independent experiments. Statistical analysis by ANOVA followed by Dunnett's test. *Denotes a statistically significantly difference to the unfortified cheese sample ($p < 0.05$)

α-glucosidase Inhibition

All three fruit digestates (goji berries, red pepper, and blackberries) demonstrated high α-glucosidase inhibition (≥ 99 % inhibition; Figure 2). Plain soft cheese (Feta and goats) had significantly greater ($p < 0.05$) α-glucosidase inhibitory properties (96.7 ± 0.9, 98.5 ± 1.3 %inhibition, respectively) compared to the RFRC hard cheese product (17.2 ± 1.1 %inhibition, Figure 2). The α-glucosidase inhibitory potential of feta cheese significantly decreased ($p < 0.05$), when combined with all three fruits, although the cheese-fruit combinations still retained a high level of inhibition (83 ± 2.5 to 93 ± 0.3 %inhibition; Figure 2, Table 2). Plain goat's cheese and its combinations were comparable in enzyme inhibitory activity (≥ 98 % inhibition) with no significant difference observed ($p > 0.05$, Table 2). RFRC had low inhibitory potential which significantly increased, when combined with all three fruits ($p < 0.05$, Figure 2, Table 2).

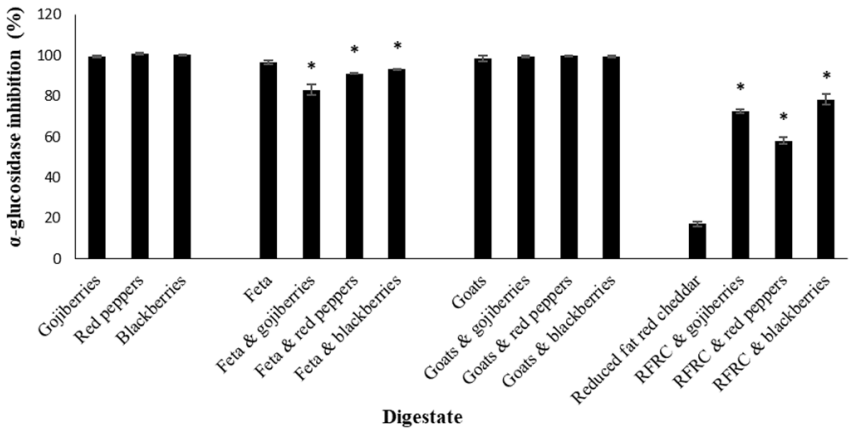


Fig.2: α-glucosidase inhibitory potential of fruit, plain cheese and cheese-fruit combinations following *in vitro* digestion. Data represents the mean ± standard deviation of at least three independent experiments. Statistical analysis by ANOVA followed by Dunnett's test. *Denotes a statistically significantly difference to the unfortified cheese sample ($p < 0.05$)

Immunomodulatory Properties

The immunomodulatory potential of the digestates was investigated by examining their ability to alter cytokine levels in Jurkat T lymphocytes. Concanavalin-A (conA) was used as a positive control as it is commonly used as a stimulant of T-cell

activation *in vitro* and has been shown to stimulate cytokine production in leukocytes.[51] All digested fruit resulted in a significant increase in TNF-α levels, compared to the positive control ($p < 0.05$, Figure 3).

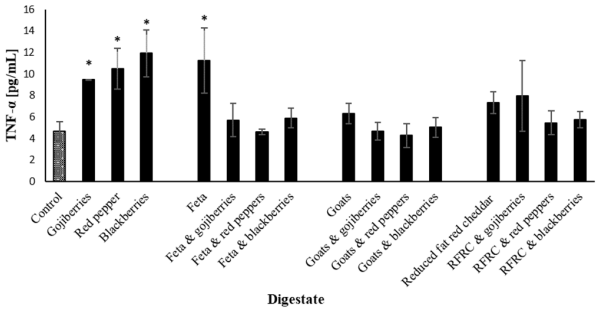


Fig.3: Effect of Digested Cheese-Fruit Combinations (0.5% v/v) on Cytokine Production in Concanavalin-A Stimulated Jurkat T Cells

Feta cheese was the only plain cheese that significantly increased TNF-α production compared to the positive control ($p < 0.05$, Figure 3). However, when comparing TNF-α secretion of the fortified cheese relative to the plain cheese, TNF-α secretion significantly reduced for feta cheese, when combined with all three fruits (Goji berries, red peppers and blackberries) ($p < 0.05$, Table 2), and there was no significant difference in TNF-α secretion observed when goat's cheese or RFRC cheese was combined with the fruit ($p > 0.05$, Table 2).

Antimicrobial Properties

None of the digestates examined in this study (individual cheese or fruit, cheese-fruit combinations) displayed antimicrobial properties against *E. coli* or *L. monocytogenes* (data not shown). However, some digestates did inhibit the growth of GBS. Penicillin G remains the first-line treatment for invasive GBS disease in adults[53] and thus, was used as a positive control in this study. Penicillin G inhibited the growth of GBS with a strong zone of inhibition (37.0 ± 1.5 mm). Regarding individual fruits, both red pepper and blackberries inhibited the growth of the GBS strain, with comparable zones of inhibition (7.7 ± 0.5, 7.0 ± 1.0 mm, respectively), however no inhibition was observed for the digested goji berries. Goat's cheese was the only plain cheese that demonstrated antimicrobial activity against GBS (8.6 ± 1.5 mm), and this was not further enhanced when the cheese and fruit were combined, with no significant difference observed ($p > 0.05$, Table 2). Plain feta cheese did not display antimicrobial activity against GBS, but when combined with red pepper and blackberries, the cheese-fruit combination exerted antimicrobial properties ($p < 0.05$, Table 2). Plain RFRC and its combinations did not display antimicrobial properties against GBS.

Discussion

Across Europe, the intake of dairy products among older adults is lower than recommended[54] and less than a quarter of older adults eat the recommended servings of fruit and vegetables per day.[55] One potential strategy to address this issue, is the use of food combinations to generate novel products; however, it is of interest to first identify the most effective combinations to ensure synergistic, rather than antagonistic effects.[56]

In this study, different cheese matrices were combined with fruit and the bioactive properties of the combinations were evaluated following *in vitro* digestion. *In vitro* antioxidant assays are frequently used to investigate the antioxidant potential of foods,[57] and as screening tools are less expensive, complex and labour intensive.[45] The reducing antioxidant properties of all cheese analyzed significantly improved when combined with the fruit ($p < 0.05$). Combining reduced fat red cheddar (RFRC) with the fruit also significantly improved the radical scavenging properties of the cheese ($p < 0.05$). Lee *et al*.,[58] also reported that the radical scavenging properties of cheddar cheese can be improved when combined with plant extracts. TPC levels were significantly lower for the majority of the cheese matrices when combined with fruit ($p < 0.05$), suggesting that phenols may not be solely responsible for the antioxidant properties observed. McDougall *et al*.,[59] observed a similar

Table 2: Comparison of the bioactive properties of cheese-fruit combinations, comparing plain cheese (control) with cheese-fruit combinations

Bioactivity		Feta	Difference from the control (95% CI)	p	Goat's cheese	Difference from the control (95% CI)	p	RFRC	Difference from the control (95% CI)	p
Antioxidant										
DPPH (% inhibition)	C	86.9 ± 5.3			89.8 ± 9.7			61.2 ± 6.0		
[1] [F(3,8) = 1.057, p = 0.419]	GB	89.7 ± 4.3	2.8 (-5.8, 11.5)	0.677	87.9 ± 1.6	-1.8 (-13.6, 9.9)	0.942	90.7 ± 1.8	29.5 (19.1, 39.8)	<0.001
[2] [F(3,8) = 1.294, p = 0.341]	RP	88.2 ± 1.0	1.3 (-7.3, 9.9)	0.946	83.4 ± 0.3	-6.4 (-18.1, 5.3)	0.333	90.6 ± 2.3	29.4 (19.0, 39.8)	<0.001
[3] [F(3,8) = 34.796, p < 0.001]	BB	91.9 ± 2.1	5.0 (-3.5, 13.7)	0.281	83.2 ± 1.8	-6.5 (-18.3, 5.2)	0.321	92.3 ± 5.6	31.1 (20.7, 41.5)	<0.001
FRAP (µmol Fe^{2+}/kg_{fw})	C	278.9 ± 4.9			718.0 ± 13.7			345.8 ± 2.7		
[1] [F(3,8) = 1580.454, p < 0.001]	GB	984.5 ± 18.6	705.6 (669.6, 741.6)	<0.001	990.9 ± 21.7	272.9 (231.1, 314.7)	<0.001	992.8 ± 17.4	646.9 (615.5, 678.4)	<0.001
[2] [F(3,8) = 176.188, p < 0.001]	RP	985.1 ± 17.1	706.2 (670.2, 742.3)	<0.001	991.4 ± 22.1	273.4 (231.6, 315.2)	<0.001	980.5 ± 13.4	634.6 (603.2, 666.1)	<0.001
[3] [F(3,8) = 1717.191, p < 0.001]	BB	977.0 ± 16.4	698.0 (662.0, 734.1)	<0.001	988.6 ± 10.4	270.6 (228.8, 312.4)	<0.001	985.3 ± 14.9	639.4 (607.9, 670.9)	<0.001
TPC (mg $GAE/100g_{fw}$)	C	379.5 ± 10.0			312.4 ± 11.0			656.4 ± 29.6		
[1] [F(3,8) = 51.337, p < 0.001]	GB	369.5 ± 15.1	-10.0 (-33.0, 12.9)	0.488	245.9 ± 11.7	-66.4 (-90.7, -42.2)	<0.001	407.9 ± 12.9	-248.5 (-289.3, 207.8)	<0.001
[2] [F(3,8) = 72.631, p < 0.001]	RP	292.3 ± 4.8	-87.2 (-110.2, -64.1)	<0.001	202.3 ± 12.0	-110.1 (-134.3, -85.8)	<0.001	404.8 ± 10.0	-251.6 (-292.4, -210.8)	<0.001
[3] [F(3,8) = 139.535, p < 0.001]	BB	324.9 ± 5.2	-54.5 (-77.6, -31.5)	<0.001	207.6 ± 4.6	-104.8 (-129.1, -80.5)	<0.001	506.3 ± 7.5	-150.1 (-190.8, -109.3)	<0.001
α-glucosidase (% Inhibition)										
[1] [F(3,8) = 52.327, p < 0.001]	C	96.7 ± 0.9			98.5 ± 1.3			17.2 ± 1.1		
[2] [F(3,8) = 1.686, p = 0.246]	GB	83.1 ± 2.5	-13.6 (-16.8, -10.3)	<0.001	99.3 ± 0.3	0.8 (-0.9, 2.6)	0.449	72.7 ± 0.9	55.4 (51.4, 59.5)	<0.001
[3] [F(3,8) = 764.858, p < 0.001]	RP	91.1 ± 0.2	-5.5 (-8.8, -2.3)	0.003	99.9 ± 0.1	1.3 (-0.4, 3.2)	0.136	58.1 ± 1.7	40.9 (36.8, 45.0)	<0.001
	BB	93.0 ± 0.3	-3.6 (-6.8, -0.3)	0.030	99.4 ± 0.6	0.9 (-0.8, 2.7)	0.374	78.2 ± 2.6	61.0 (56.9, 65.1)	<0.001
Immunomodulatory (pg/mL)										
[1] [F(3,8) = 8.477, p = 0.007]	C	11.2 ± 3.0			6.3 ± 0.9			7.3 ± 1.0		
[2] [F(3,8) = 2.587, p = 0.126]	GB	5.7 ± 1.5	-5.5 (-9.6, -1.3)	0.013	4.6 ± 0.8	-1.6 (-3.8, 0.6)	0.157	7.9 ± 3.3	0.6 (-3.7, 4.9)	0.953
[3] [F(3,8) = 1.272, p = 0.348]	RP	4.6 ± 0.2	-6.6 (-10.7, -2.4)	0.005	4.2 ± 1.1	-2.0 (-4.2, 0.1)	0.073	5.4 ± 1.0	-1.8 (-6.2, 2.4)	0.501
	BB	5.8 ± 0.9	-5.3 (-9.4, -1.1)	0.015	5.0 ± 0.9	-1.3 (-3.5, 0.9)	0.286	5.7 ± 0.7	-1.5 (-5.9, 2.7)	0.623
Antimicrobial										
(Zone of inhibition, mm)										
[1] [F(3,8) = 37.400, p < 0.001]	C	0.0 ± 0.0			8.6 ± 1.5			-		
[2] [F(3,8) = 2.295, p = 0.155]	GB	0.0 ± 0.0	0.0 (-2.6, 2.6)	1.000	6.0 ± 1.0	-2.6 (-6.1, 0.7)	0.133	-	-	-
[3] -	RP	5.0 ± 1.0	5.0 (2.3, 7.6)	0.002	6.3 ± 2.0	-2.3 (-5.7, 1.1)	0.199	-	-	-
	BB	8.0 ± 2.0	8.0 (5.3, 10.6)	<0.001	8.0 ± 1.0	-0.6 (-4.1, 2.7)	0.900	-	-	-

Values represent mean ± standard deviation of at least three independent experiments. p-values of the difference from control (Dunnett test).

[1], [2] and [3] Analysis of variance (ANOVA) results for Feta, Goat's cheese and RFRC, respectively. "-" Denotes no antimicrobial properties.

RFRC: Reduced fat red cheddar; C: Control (Plain cheese); GB: Goji berries; RP: Red pepper; BB: Blackberries.

effect when a dairy matrix (ice-cream) was combined with raspberries.[59] A decrease in TPC levels may be due to reduced enzyme secretion in an older adult digestive system and the impact this has on protein-polyphenol interactions. While some interactions can prevent phenolic compounds from being degraded, others may hinder their release from a food matrix.[60]

The incidence of type II diabetes increases with age.[61] One antidiabetic strategy is to inhibit key enzymes relevant to glucose metabolism.[62] In this study, the α-glucosidase inhibitory potential of cheese-fruit combinations were investigated, and all digestates displayed high inhibitory potential (≥58% inhibition). Similar to Apostolidis *et al*.,[33] this study demonstrated that cheese enriched with berries inhibited α-glucosidase, however, results from this study also demonstrated that this property was retained following *in vitro* digestion. Berries have been shown to be effective α-glucosidase inhibitors, largely due to their tannin content.[63] RFRC combined with all fruit significantly improved the enzyme inhibitory properties of the cheese ($p < 0.05$). However, the α-glucosidase inhibitory potential of feta cheese significantly decreased when combined with the fruit, suggesting potential antagonistic effects ($p < 0.05$). The antagonistic effects observed may be due to specific protein-polyphenol interactions in the feta-fruit combinations.[64] Ni *et al*.,[65] observed similar effects when combining a soft dairy matrix (yogurt) with berries, which reduced the antidiabetic properties of the yogurt.

Aged-related changes in the immune system include cytokine dysregulation.[66] Tumor necrosis factor-α (TNF-α) is an important immune regulator[67] that has both anti-inflammatory[68] and pro-inflammatory properties.[69] Abnormal levels of TNF-α are associated with conditions such as rheumatoid arthritis, Crohn's disease, atherosclerosis, psoriasis, sepsis, diabetes, and obesity.[69] Aging is associated with elevated levels of TNF-α,[68] thus food-based strategies that regulate levels of this important immunomodulatory agent could help modulate the immune response in older adults. The immunomodulatory properties of feta cheese were significantly altered following enrichment with fruit, with a reduction in TNF-α secretion observed ($p < 0.05$). This may be due to a combination of bioactive compounds in the cheese-fruit mixture, including anti-inflammatory compounds that can be found in fruit[40–42] and conjugated linoleic acids (CLA) in cheese. Cheese made from sheep's milk is naturally rich in CLA and has been associated with anti-inflammatory properties.[70] López-García *et al*.,[71] investigated the anti-inflammatory effects of a sterol enriched milk-based fruit beverage and found that the beverage demonstrated moderate anti-inflammatory effects and suggested that a combination of bioactive compounds may be responsible for the effect observed.

Aging is also associated with an increased susceptibility to infection.[3] Group B *Streptococci* (GBS) infections are increasing in older adult populations,[53,72,73] with serotype V being the most prevalent serogroup associated with invasive disease in adults.[74] The gastrointestinal tract is a reservoir for GBS[75] and colonization of the gut is considered a first crucial step in the progression of infections.[76] Antimicrobial peptides have been previously identified in cheese[20,21] and fruit.[77,78] Interestingly, feta cheese was not active against GBS, until combined with fruit suggesting potential synergistic effects.[79,80] Digested hard cheese (cheddar) displayed no bactericidal effect, even when combined with the fruit. Fang *et al*.,[38] confirmed that soft cheese matrices are easily disrupted during gastric digestion, with a fast release of peptides compared to harder cheese matrices, which may be linked to the lack of antimicrobial activity of the RFRC product. Further studies are required to confirm the antimicrobial properties of the cheese-fruit combinations against other GBS strains and serotypes, but these preliminary studies suggest that a diet-based strategy to reduce GBS colonization *in vivo* and limit GBS infections in older adults warrants further investigation.[53]

It is important to acknowledge that this study was based on *in vitro* investigations and further studies would be necessary to confirm *in vivo* effects. In addition, future work could identify the compounds responsible for the activities observed. The current study adds to existing research that supports combining cheese with other food matrices, such as fruit, to enhance the bioactive properties of cheese. Understanding the potential synergistic and/or antagonistic effects that can occur when dairy and fruit matrices are combined could help aid the design of novel functional foods for older adults.

Conclusion

To date, studies reporting on the synergistic effects observed when cheese and fruit are combined are limited, and no studies to date have examined these effects following *in vitro* digestion with an older adult gut model. Certain cheese products

combined with fruit significantly improved the antioxidant, α-glucosidase inhibitory, antimicrobial, and immunomodulatory properties of the cheese. In particular, the antioxidant and α-glucosidase inhibitory potential of reduced fat red cheddar significantly improved when combined with fruit. Cheddar cheese is a product that is well received by older adults, who also have a preference towards reduced fat products and low-calorie intake. Novel RFRC-fruit combinations could be an attractive functional food option for this cohort.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Lunenfeld B., Stratton P. The clinical consequences of an ageing world and preventive strategies. *Best Pract Res Clin Obstet Gynaecol*. 2013;27(5):643-659. doi:10.1016/J.BPOBGYN.2013.02.005
2. Jaul E., Barron J. Age-Related Diseases and Clinical and Public Health Implications for the 85 Years Old and Over Population. *Front public Heal*. 2017;5:335. doi:10.3389/fpubh.2017.00335
3. Rescigno T., Micolucci L., Tecce MF., Capasso A. Bioactive Nutrients and Nutrigenomics in Age-Related Diseases. *Molecules*. 2017;22(1):105. doi:10.3390/molecules22010105
4. Shlisky J., Bloom DE., Beaudreault AR., et al. Nutritional considerations for healthy aging and reduction in age-related chronic disease. *Adv Nutr An Int Rev J.* 2017;8(1):17-26. doi:10.3945/an.116.013474
5. Everitt A V., Hilmer SN., Brand-Miller JC., *et al*. Dietary approaches that delay age-related diseases. *Clin Interv Aging.* 2006;1(1):11-31. doi:10.2147/ciia.2006.1.1.11
6. Eussen S., Klungel O., Garssen J., *et al*. Support of drug therapy using functional foods and dietary supplements: Focus on statin therapy. *Br J Nutr*. 2010;103(9):1260-1277. doi:10.1017/S0007114509993230
7. Scolaro B., Soo Jin Kim H., de Castro IA. Bioactive compounds as an alternative for drug co-therapy: Overcoming challenges in cardiovascular disease prevention. *Crit Rev Food Sci Nutr.* 2018;58(6):958-971. doi:10.1080/10408398.2016.1235546
8. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr*. 2003;78(3):517S-520S. doi:10.1093/ajcn/78.3.517S
9. Food Safety Authority of Ireland. Scientific Recommendations for Healthy Eating Guidelines in Ireland. *FSAI*;2011:1-92. https://www.fsai.ie/WorkArea/DownloadAsset.aspx?id=16765. Accessed Janurary 11, 2019.
10. Food and Agriculture Organization of the United Nations. Food-based dietary guidelines - Ireland. FAO;2012. http://www.fao.org/nutrition/education/food-dietary-guidelines/regions/countries/ireland/en/. Accessed January 11, 2019.
11. Slavin JL., Lloyd B. Health Benefits of Fruits and Vegetables. *Adv Nutr*. 2012;3(4):506-516. doi:10.3945/an.112.002154
12. Kadell A., Nicklett E. Fruit and vegetable intake among older adults. *Maturitas*. 2013;75(4):305-312. doi:10.1016/j.maturitas.2013.05.005
13. Thorning TK., Raben A., Tholstrup T., Soedamah-Muthu SS., Givens I., Astrup A. Milk and dairy products: Good or bad for human health? An assessment of the totality of scientific evidence. *Food Nutr Res*. 2016;60:32527. doi:10.3402/fnr.v60.32527
14. Rene R. Dairy products, yogurts, and bone health. *Am J Clin Nutr*. 2014;99(5):1256S-1262S. doi:10.3945/ajcn.113.073056
15. Korhonen H. Milk-derived bioactive peptides: From science to applications. *J Funct*

Foods. 2009;1(2):177-187. doi:10.1016/j.jff.2009.01.007

16. Park YW., Nam MS. Bioactive peptides in milk and dairy products: a review. *Korean J food Sci Anim Resour*. 2015;35(6):831-840. doi:10.5851/kosfa.2015.35.6.831
17. Sluijs I., Forouhi G N., Beulens WJ J., *et al*. The amount and type of dairy product intake and incident type 2 diabetes: Results from the EPIC-InterAct Study. *Am J Clin Nutr*. 2012;96(2):382-390. doi:10.3945/ajcn.111.021907
18. Wang P-Y., Fang J-C., Gao Z-H., Zhang C., Xie S-Y. Higher intake of fruits, vegetables or their fiber reduces the risk of type 2 diabetes: A meta-analysis. *J Diabetes Investig*. 2016;7(1):56-69. doi:10.1111/jdi.12376
19. Rizzello CG., Losito I., Gobbetti M., Carbonara T., De Bari MD., Zambonin PG. Antibacterial activities of peptides from the water-soluble extracts of Italian cheese varieties. *J Dairy Sci*. 2005;88(7):2348-2360. doi:10.3168/jds.S0022-0302(05)72913-1
20. Losito I., Carbonara T., De Bari MD., *et al*. Identification of peptides in antimicrobial fractions of cheese extracts by electrospray ionization ion trap mass spectrometry coupled to a two-dimensional liquid chromatographic separation. *Rapid Commun Mass Spectrom*. 2006;20(3):447-455. doi:10.1002/rcm.2323
21. Pritchard SR., Phillips M., Kailasapathy K. Identification of bioactive peptides in commercial Cheddar cheese. *Food Res Int*. 2010;43(5):1545-1548. doi:10.1016/j.foodres.2010.03.007
22. Mohanty DP., Mohapatra S., Misra S., Sahu PS. Milk derived bioactive peptides and their impact on human health – A review. *Saudi J Biol Sci.* 2016;23(5):577-583. doi:10.1016/j.sjbs.2015.06.005
23. Rahmawati IS., Suntornsuk W. Effects of Fermentation and Storage on Bioactive Activities in Milks and Yoghurts. *Procedia Chem*. 2016;18:53-62. doi:10.1016/j.proche.2016.01.010
24. Cavanagh HMA., Hipwell M., Wilkinson JM. Antibacterial activity of berry fruits used for culinary purposes. *J Med Food.* 2003;6(1):57-61. doi:10.1089/109662003765184750
25. O'Mahony R. The antibacterial properties of dietary fruit. In: Watson RR., Preedy VR. Bioactive foods in promoting health. Elsevier Inc; 2010:147-166 https://www.elsevier.com/books/bioactive-foods-in-promoting-health/watson/978-0-12-374628-3. Accessed April 30, 2019.
26. Meneguetti BT., Machado L dos S., Oshiro KGN., Nogueira ML., Carvalho CME., Franco OL. Antimicrobial peptides from fruits and their potential use as biotechnological Tools-A review and outlook. *Front Microbiol*. 2016;7:2136. doi:10.3389/fmicb.2016.02136
27. Mcgarrigle C., Donoghue O., Scarlett S., *et al*. Health and Wellbeing: Active Ageing for Older Adults in Ireland. Evidence from The Irish Longitudinal Study on Ageing. 2017:1-218. https://tilda.tcd.ie/publications/reports/pdf/w3-key-findings-report/TILDA%20Wave%203%20Key%20Findings%20report.pdf. Accessed March 10, 2020.
28. Fernandez MA., Marette A.. Potential Health Benefits of Combining Yogurt and Fruits Based on Their Probiotic and Prebiotic Properties. *Adv Nutr An Int Rev J*. 2017;8(1):155S-164S. doi:10.3945/an.115.011114
29. Jacobs DR., Gross MD., Tapsell LC., Tapsell LC. Food synergy: an operational concept for understanding nutrition. *Am J Clin Nutr.* 2009;89(5):1543S-1548S. doi:10.3945/ajcn.2009.26736B
30. Maurya VK. Factors influencing the absorption of vitamin D in GIT : an overview. *J Food Sci Technol*. 2017;54(12):3753-3765. doi:10.1007/s13197-017-2840-0
31. Wang S., Meckling KA., Marcone MF., Kakuda Y., Tsao R. Synergistic, additive, and antagonistic effects of food mixtures on total antioxidant capacities. *J Agric Food Chem.* 2011;59(3):960-968. doi:10.1021/jf1040977
32. Al-Otaibi MM., Haddadin JS., Haddadin MSY. Mold-ripened soft cheeses fortified with date palm fruit product as functional dairy products. *Pakistan J Biol Sci*. 2016;19(1):11-25. doi:10.3923/pjbs.2016.11.25
33. Apostolidis E., Kwon YI., Shetty K. Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. *Innov Food Sci Emerg Technol*. 2007;8(1):46-54. doi:10.1016/j.ifset.2006.06.001
34. Khalifa SA., Wahdan KM. Improving the

quality characteristics of white soft cheese using cranberry (Vaccinium macrocarpon) fruit extract. *Int Food Res J*. 2015;22(6):2203-2211. http://www.ifrj.upm.edu.my/22%20(06)%202015/(4).pdf

35. Rashidinejad A., Birch EJ., Everett DW. Antioxidant activity and recovery of green tea catechins in full-fat cheese following gastrointestinal simulated digestion. *J Food Compos Anal*. 2016;48:13-24. doi:10.1016/j.jfca.2016.02.004
36. Linnan MJ., Mascola L., Lou XD., *et al*. Epidemic listeriosis associated with Mexican-style cheese. *N Engl J Med.* 1988;319(13):823-828. doi:10.1056/NEJM198809293191303
37. Hayes K., Cotter L., Barry L., O'Halloran F. Emergence of the L phenotype in Group B Streptococci in the South of Ireland. *Epidemiol Infect*. 2017;145(16):3535-3542. doi:10.1017/S0950268817002461
38. Fang X., Rioux LE., Labrie S., Turgeon SL. Commercial cheeses with different texture have different disintegration and protein/peptide release rates during simulated *in vitro* digestion. *Int Dairy J*. 2016;56:169-178. doi:10.1016/j.idairyj.2016.01.023
39. Tavares L., Figueira I., McDougall GJ., *et al*. Neuroprotective effects of digested polyphenols from wild blackberry species. *Eur J Nutr*. 2013;52(1):225-236. doi:10.1007/s00394-012-0307-7
40. Chávez-Mendoza C., Sanchez E., Muñoz-Marquez E., Sida-Arreola JP., Flores-Cordova MA. Bioactive compounds and antioxidant activity in different grafted varieties of bell pepper. *Antioxidants*. 2015;4(2):427-446. doi:10.3390/antiox4020427
41. Skrovankova S., Sumczynski D., Mlcek J., Jurikova T., Sochor J. Bioactive Compounds and Antioxidant Activity in Different Types of Berries. *Int J Mol Sci*. 2015;16(10):24673-24706. doi:10.3390/ijms161024673
42. Vulić JJ., Čanadanović-Brunet JM., Ćetković GS., Djilas SM., Tumbas Šaponjac VT., Stajčić SS. Bioactive Compounds and Antioxidant Properties of Goji fruits (*Lycium barbarum* L.) Cultivated in Serbia. *J Am Coll Nutr*. 2016;35(8):692-698. doi:10.1080/07315724.2016.1142404
43. Plante AM., McCarthy AL., O'Halloran F. Cheese as a functional food for older adults: comparing the bioactive properties of different cheese matrices following simulated gastrointestinal *in vitro* digestion. *Int J Food Sci Nutr*. 2020:1-14. doi:10.1080/09637486.2020.1825644
44. Minekus M., Alminger M., Alvito P., *et al*. A standardised static *in vitro* digestion method suitable for food – an international consensus. *Food Funct Food Funct*. 2014;5(5):1113-1124. doi:10.1039/c3fo60702j
45. López-Alarcón C., Denicola A. Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. *Anal Chim Acta*. 2013;763:1-10. doi:10.1016/J.ACA.2012.11.051
46. Brand-Williams W., Cuvelier ME., Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci Technol.* 1995;28(1):25-30. doi:10.1016/S0023-6438(95)80008-5
47. Benzie IF., Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol*. 1999;299:15-27. doi: 10.1016/s0076-6879(99)99005-5.
48. Singleton VL., Rossi JA., JR R. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic.* 1965;16(3):144-158. https://www.ajevonline.org/content/16/3/144.
49. Derosa G., Maffioli P. α-Glucosidase inhibitors and their use in clinical practice. *Arch Med Sci*. 2012;8(5):899-906. doi:10.5114/aoms.2012.31621
50. Gabrani R., Jain R., Sharma A., Sarethy I., Dang S., Gupta S. Antiproliferative effect of Solanum nigrum on human leukemic cell lines. *Indian J Pharm Sci.* 2012;74(5):451-453. doi:10.4103/0250-474X.108421
51. McCarthy AL., O'Callaghan YC., Connolly A., Piggott CO., FitzGerald RJ., O'Brien NM. A study of the ability of bioactive extracts from brewers' spent grain to enhance the antioxidant and immunomodulatory potential of food formulations following *in vitro* digestion. *Int J Food Sci Nutr*. 2015;66(2):230-235. doi:10.3109/09637486.2014.979314

52. Meira SMM., Daroit DJ., Helfer VE., *et al*. Bioactive peptides in water-soluble extracts of ovine cheeses from Southern Brazil and Uruguay. *Food Res Int.* 2012;48(1):322-329. doi:10.1016/J.FOODRES.2012.05.009
53. High KP., Edwards MS., Baker CJ. Group B Streptococcal Infections in Elderly Adults. *Clin Infect Dis.* 2005;41(6):839-847. doi:10.1086/432804
54. Ribeiro I., Gomes M., Figueiredo D., Lourenço J., Paúl C., Costa E. Dairy Product Intake in Older Adults across Europe Based On the SHARE Database. *J Nutr Gerontol Geriatr*. 2019;38(3):297-306. doi:10.1080/21551197.2019.1627972
55. Baker AH., Wardle J. Sex differences in fruit and vegetable intake in older adults. *Appetite*. 2003;40(3):269-275. doi:10.1016/S0195-6663(03)00014-X
56. Tapsell LC., Neale EP., Satija A., Hu FB. Foods, Nutrients, and Dietary Patterns: Interconnections and Implications for Dietary Guidelines. *Adv Nutr*. 2016;7(3):445-454. doi:10.3945/an.115.011718
57. Huang D., Boxin OU., Prior RL. The chemistry behind antioxidant capacity assays. *J Agric Food Chem*. 2005;53(6):1841-1856. doi:10.1021/jf030723c
58. Lee N-K., Jeewanthi RKC., Park E-H., Paik H-D. Short communication: Physicochemical and antioxidant properties of Cheddar-type cheese fortified with Inula britannica extract. *J Dairy Sci*. 2015;99(1):83-88. doi:10.3168/jds.2015-9935
59. McDougall GJ., Dobson P., Smith P., Blake A., Stewart D. Assessing potential bioavailability of raspberry anthocyanins using an *in vitro* digestion system. *J Agric Food Chem.* 2005;53(15):5896-5904. doi:10.1021/jf050131p
60. Ribas-Agustí A., Martín-Belloso O., Soliva-Fortuny R., Elez-Martínez P. Food processing strategies to enhance phenolic compounds bioaccessibility and bioavailability in plant-based foods. *Crit Rev Food Sci Nutr*. 2018;58(15):2531-2548. doi:10.1080/10408398.2017.1331200
61. Kalyani RR., Golden SH., Cefalu WT. Diabetes and aging: Unique considerations and goals of care. *Diabetes Care.* 2017;40(4):440-443. doi:10.2337/dci17-0005
62. Kirkman MS., Briscoe VJ., Clark N., *et al.* Diabetes in older adults. *Diabetes Care*. 2012;35(12):2650-2664. doi:10.2337/dc12-1801
63. Xiao J., Kai G., Yamamoto K., Chen X. Advance in dietary polyphenols as α-Glucosidases inhibitors: a review on structure-activity relationship aspect. *Crit Rev Food Sci Nutr*. 2013;53(8):818-836. doi:10.1080/10408398.2011.561379
64. Yildirim-Elikoglu S., Erdem YK. Interactions between milk proteins and polyphenols: Binding mechanisms, related changes, and the future trends in the dairy industry. *Food Rev Int*. 2018;34(7):665-697. doi:10.1080/87559129.2017.1377225
65. Ni H., Hayes HE., Stead D., Raikos V. Incorporating salal berry (Gaultheria shallon) and blackcurrant (Ribes nigrum) pomace in yogurt for the development of a beverage with antidiabetic properties. Heliyon. 2018;4(10):e00875. doi:10.1016/j.heliyon.2018.e00875
66. Brüünsgaard H., Pedersen BK. Age-related inflammatory cytokines and disease. *Immunol Allergy Clin North Am*. 2003;23(1):15-39. doi:10.1016/S0889-8561(02)00056-5
67. Rieckmann P., Tuscano JM., Kehrl JH. Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6) in B-Lymphocyte Function. *Methods*. 1997;11(1):128-132. doi:10.1006/METH.1996.0396
68. Bruunsgaard H., Pedersen M., Pedersen BK. Aging and proinflammatory cytokines. *Curr Opin Hematol*. 2001;8(3):131-136. doi:10.1097/00062752-200105000-00001
69. Parameswaran N., Patial S. Tumor necrosis factor-α signaling in macrophages. *Crit Rev Eukaryot Gene Expr*. 2010;20(2):87-103. doi:10.1615/critreveukargeneexpr.v20.i2.10
70. Sofi F., Buccioni A., Cesari F., *et al*. Effects of a dairy product (pecorino cheese) naturally rich in cis-9, trans-11 conjugated linoleic acid on lipid, inflammatory and haemorheological variables: A dietary intervention study. *Nutr Metab Cardiovasc Dis*. 2010;20(2):117-124. doi:10.1016/j.numecd.2009.03.004
71. López-García G., Cilla A., Barberá R., Alegría A., Recio MC. Effect of a milk-based fruit beverage enriched with plant sterols and/or galactooligosaccharides in a murine

chronic colitis model. *Foods.* 2019;8(4):114. doi:10.3390/foods8040114

72. Phares CR., Lynfield R., Farley MM., et al. Epidemiology of Invasive Group B Streptococcal Disease in the United States, 1999-2005. *JAMA*. 2008;299(17):2056-2065. doi:10.1001/jama.299.17.2056
73. Kothari NJ., Morin CA., Glennen A., *et al*. Invasive group B streptococcal disease in the elderly, Minnesota, USA, 2003-2007. *Emerg Infect Dis*. 2009;15(8):1279-1281. doi:10.3201/eid1508.081381
74. Teatero S., McGeer A., Low DE., *et al*. Characterization of invasive group B streptococcus strains from the greater Toronto area, Canada. *J Clin Microbiol*. 2014;52(5):1441-1447. doi:10.1128/JCM.03554-13
75. Spencer BL., Deng L., Patras KA., *et al.* Cas9 contributes to group b streptococcal colonization and disease. *Front Microbiol*. 2019;10:1930. doi:10.3389/fmicb.2019.01930
76. Shabayek S., Spellerberg B. Group B streptococcal colonization, molecular characteristics, and epidemiology. *Front Microbiol*. 2018;9:437. doi:10.3389/fmicb.2018.00437
77. Guzmán-Rodríguez JJ., López-Gómez R., Suárez-Rodríguez LM., *et al*. Antibacterial activity of defensin PaDef from avocado fruit (Persea americana var. drymifolia) expressed in endothelial cells against escherichia coli and staphylococcus aureus. *Biomed Res Int*. 2013;2013. doi:10.1155/2013/986273
78. Seo HH., Park S., Park S., et al. Overexpression of a defensin enhances resistance to a fruit-specific anthracnose fungus in pepper. *PLoS One*. 2014;9(5):e97936. doi:10.1371/journal.pone.0097936
79. Clare D., Catignani G., Swaisgood H. Biodefense Properties of Milk: The Role of Antimicrobial Proteins and Peptides. *Curr Pharm Des.* 2005;9(16):1239-1255. doi:10.2174/1381612033454874
80. Hayes M., Stanton C., Fitzgerald GF., Ross RP. Putting microbes to work: Diary fermentation, cell factories and bioactive peptides. Part II: *Bioactive peptide functions. Biotechnol J*. 2007;2(4):435-449. doi:10.1002/biot.200700045