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Reformulation and food combination as strategies to modulate glycaemia: the case of apple pomace containing biscuits administered with apple juice to healthy rats

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Abstract

Conventional (CB) and apple-pomace-reformulated (RB) biscuits were administered to healthy rats. Although the areas under curve (AUC) of glucose concentration were comparable between samples, differences in the glycaemic profile of CB and RB were observed. RB caused an initial steeper increase in glycaemia but a shift in the glycaemic peak from 45 to 60 min, as compared to CB. When CB or RB was ingested with apple juice (AJ) no differences were observed as compared to their ingestion with a soft drink (SD) simulating AJ sugar content, indicating that reformulation, more than the presence of AJ, was crucial in affecting the glycaemic response. Consumer acceptability towards reformulation was assessed through conjoint analysis, by simulating labels reporting information on reformulation. Consumers preferred information generally referring to the health-promoting effect (i.e. “low sugar” and “high fibre” contents), despite directly relating to a specific disease (i.e. “suitable for diabetics” and “low glycaemic index”).

Keywords: Functional food; glycaemic response; bioactive compounds; polyphenols; dietary intervention; food choice

Introduction

In the last decades the incidence of type 2 diabetes has dramatically increased (WHO 2016) and one of its major causes is obesity (American Diabetes Association 2004). Since the drugs for its treatment often lead side effects (Cheng and Fantus 2005), the need for alternative solutions has become impellent (Ríos et al. 2015). As an increase in physical activity and a change in dietary habits were demonstrated to play a role towards the prevention and management of type 2 diabetes (WHO 2003), in the last years the research interest in diet-related interventions has undergone a steep increase. In fact, the glycaemic index of a variety of commercial foods has been measured (Foster-Powell et al. 2002; Aston et al. 2008; Scazzina et al. 2016). Still, the literature relevant to the effect of technological interventions, intended as formulation and processing, on food ability to modulate the glycaemic response is not exhaustive and provides uncertain indications (Ferrer-Mairal et al. 2012; CS Brennan et al. 1996; Anderson et al. 2010; Englyst et al. 1996; Englyst et al. 1999; Ren et al. 2016; Garsetti et al. 2005; Milek Dos Santos et al. 2014; Monro et al. 2010; Al-Mssallem et al. 2011). Nonetheless, several tested foods positively affected the glycaemic response upon both *in vitro* and *in vivo* trials. However, neither the mechanisms nor the molecules underlying these effects have been clearly elucidated yet. Thus, functional foods (i.e. foods providing a health benefit beyond basic nutrition) oriented towards glycaemic control have been developed and new ingredients and formulations are under investigation (Martinez-Saez et al. 2017; De Oliveira Lopes et al. 2019). Most of the products available on the market are claimed as “low sugar”, “sugar-free”, “no added sugar”, “source of fibre”, “high fibre”, accordingly with the Reg. (EC) No 1924/2006, instead of reporting the indication “low GI”. To this regard, the EFSA stated that is not possible to claim the ability of a food in inducing a low or reduced glycaemic response as the scientific substantiation is not exhaustive to establish a cause-effect relationship between the consumption of certain products and a positive effect on the glycaemic response (EFSA Panel on Dietetic

Products Nutrition and Allergies (NDA)2010). Thus, within the list of admitted health claims reported in Reg. (EU) No 432/2012, those relevant to the impact of food consumption on the glycaemic response refer only to the presence of dietary fibre, whereas no indication on the impact of other bioactive compounds on glycaemic response is reported.

Such missing information is attributable to the lack of rapid and cheap methods for assessing the glycaemic index of foods, as well as to the absence of evidence on the cause-effect relationship between glycaemic index/response and food composition.

Consequently, despite the huge amount of technological interventions that have been developed recently, their potential effect on the glycaemic index of foods remains unravelled. In fact, the antidiabetic property is generally attributed to the presence of single bioactive compounds, mainly derived from plant extracts, among which are in particular phenolic compounds (Del Rio et al. 2013; Meng et al. 2013; Kim 2015; Coe and Ryan 2016). However, the antidiabetic efficacy is mostly studied in simplified systems containing the bioactive whereas the matrix complexity is barely considered (Ali et al. 2016; Kim et al. 2016; Tarko and Duda-Chodak 2020). Consequently, these researches do not take into consideration the role of the food system nor the possible interactions between the bioactive molecules and other food compounds. Even less investigated is the effect of meal consumption on the glycaemic response. In fact, although a bioactive compound can be ingested within a functional food, its bioactivity could be affected by the co-ingestion with different food in a meal (McClements et al. 2015).

Besides demonstrating the efficacy of reformulation, a crucial issue to be addressed is the effective communication of the health benefits delivered through such interventions. To this regard, labelled information, especially nutritional and health claims, indeed affect the reaction of consumers towards the consumption of a new product, thus contributing to its success (Lähteenmäki 2013). Since getting an insight into these aspects is crucial for manufacturers to successfully deliver the product on the market, consumer research is applied during testing and launching. In particular, the efficacy of nutrition and health claims can be assessed through different tools, among which is conjoint analysis (Van Kleef et al. 2005).

In light of these considerations, the aim of this study was to assess the glycaemic response of healthy rats being administered with biscuits, which were reformulated by partially replacing wheat flour with apple pomace. This is discarded during apple juice production and accounts for almost 10 million tons/year, i.e. the 25% of apple mass (Wolfe and Liu 2003). Generally, it is disposed of or used to produce energy (Rodríguez et al. 2006). However, being still rich in bioactives, such as dietary fibre and phenolic compounds (Sudha et al. 2007), its reuse as a functional ingredient might be a promising approach to reduce the glycaemic response of foods.

Conventional and reformulated biscuits were administered alone or in combination with apple juice or a soft drink simulating apple juice sugar content, and the glycaemic response was evaluated. Finally, the consumer response towards reformulation was assessed by conjoint analysis.

Materials and methods

Sample preparation

Commercial clear apple juice (Skipper-Zuegg, Verona, Italy) was purchased on the local market. A soft drink mimicking the sugar composition of apple juice, i.e. 57.3 mg/mL fructose, 26.3 mg/mL glucose and 12.6 mg/mL sucrose, was prepared (USDA 2019).

Conventional (CB) and reformulated (RB) biscuits were obtained as reported by Alongi et al. (2019). Briefly, CB were prepared by mixing wheat flour (51.6%, w/w), egg (20.7%, w/w), sucrose (17.2%, w/w), sunflower oil (8.6%, w/w), baking powder (1.7%, w/w), and NaCl (0.2%, w/w). After resting for 30 min at 4 °C, the dough was shaped in 2-mm layer and 50-mm diameter discs, which were baked at 140 °C for 15 min. RB were obtained by partially replacing (20%, w/w) wheat flour in CB formulation with the powder (particle size < 200 μm) obtained upon vacuum drying (75 °C and 0.1 MPa) of the apple pomace recovered during juice extraction.

Extraction and purification

Powdered CB (4.0 g) and RB (4.0 g), apple pomace (AP, 1.0 g) and commercial clear apple juice (AJ, 10 mL) samples were spiked with a methanolic solution of 3-hydroxycinnamic acid (50 μg/mL) as internal standard. The AJ sample was directly lyophilised while the other samples were extracted in H_2O containing 0.2% formic acid (45 mL) under magnetic stirring for 3 h at room temperature, followed by centrifugation at 5000 rpm for 8 min. The supernatants were filtered, collected in a glass tube and finally lyophilised. The dry samples were then dissolved in H_2O with 0.2% formic acid (2 mL) and subjected to SPE purification.

For this purpose, each sample was loaded on a C18 SPE column pre-conditioned by sequentially passing 5 mL of MeOH with 0.2% formic acid and 5 mL of H_2O with 0.2% formic acid. After loading the sample, the column was washed with 10 mL of H_2O containing 0.2% formic acid, and the phenolic fraction was eluted with 5 mL of MeOH with 0.2% formic acid. The solvents were removed under vacuum, and the residue was dissolved in 1 mL of H_2O/MeOH (9:1, v/v) for the HPLC-DAD-ESI-MS^n analysis.

Phenolic composition

Phenolic identification and quantification were determined according to our previous published work (Alongi et al. 2018). Briefly, chromatographic analysis was performed with a Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA) equipped with a C18 column (Synergi Hydro, 4 µm, 250 × 2.0 mm, Phenomenex, Italy), using H_2O containing 0.2% formic acid and MeOH with 0.2% formic acid as mobile phases. The UHPLC system was coupled with an Ultimate 3000 RS Diode Array detector (Thermo Scientific, San Jose, CA, USA) and a Finnigan LXQ linear trap mass spectrometer (Thermo Scientific, San Jose, CA, USA), fitted with an ESI source operating in negative mode, in parallel by splitting the mobile phase 1:1 (HPLC-DAD-ESI-MSn). Phenolic compounds were identified by their MS2 fragmentation pattern and the quantitative analysis was performed in the range of 200–400 nm. Chromatograms were recorded at 280 nm for (+)-catechin, (−)-epicatechin, procyanidin B2, epigallocatechin gallate, 3-hydroxycinnamic acid (I.S.), phloretin-xyloglucoside, phloridzin, and phloretin, 314 nm for 4-p-coumaroylquinic acid, 328 nm for chlorogenic acid, and 258 nm for quercetin-3-O-galactoside, quercetin-3-O-xyloside, quercetin-3-O-arabinoside and quercetin-3-O-rhamnoside, respectively. Calibration curves ($R^2 >$ 0.99) were prepared by diluting a stock solution of each standard in a 9:1 ratio of H_2O/MeOH (v/v) with 0.2% of formic acid in the range of 12–3000 ng/mL with a constant concentration of the I.S. (500 ng/mL).

Experimental animals

Young adult (six-week-old) male Wistar rats (*Rattus norvegicus*, $n = 20$) weighing 242 ± 12 g were obtained from Envigo RMS Srl (San Pietro al Natisone, Italy). They were housed in wire-bottomed cages in a room with controlled temperature (25 °C) and lighting (12 h light/dark cycle) and had free access to water and to a commercial rodent diet (Envigo RMS Srl, San Pietro al Natisone, Italy) for 1 week. All procedures were carried out according to the guidelines enforced in Italy and in compliance with the guide of the National Research Council, upon approval by the Italian Committee for Bioethics (D. Lgs 116/1992; National Research Council 2011).

Experimental procedure

After 15 h fasting, samples were intragastrical administered to rats using a syringe coupled with a cannula. The rat was placed in vertical position and the cannula was introduced through the pharynx. Experiments were carried out to test the effects of technological and dietary interventions by administering each treatment to a group of five rats. Rats were randomly assigned to the groups and a first set of 4 conditions was tested. To minimise the number of animals employed, rats were further kept under the conditions reported in Paragraph 2.4 for 2 weeks, and after this time the second set of treatments was assigned to some groups.

The tested effects and treatments are reported in Table 1. In particular, a glucose solution was administered in a dose providing 1.58 g/kg_{bw} glucose, which is the concentration recommended to test the glycaemic response in rat models (Belobrajdic et al. 2016). The dose was adapted to the weight of each rat, which was measured immediately before the beginning of each experiment.

Table 1. Treatments to test the effects of technological and dietary interventions on the glycaemic response of rats, and their composition in terms of total dietary fibre (TDF), sugar (fructose, FRU; glucose, GLU; sucrose, SUC), total available carbohydrate (TAC) and total phenolic (TPC) contents.

Tested effect	Treatment	TDF (%)	FRU (%)	GLU (%)	SUC (%)	TAC (%)	TPC (%)
Control	Glucose	–	–	100	–	–	–
Technological intervention	Conventional biscuit (CB)	1.64	0.00	0.09	20.25	72.42	0.05
	Reformulated biscuit (RB)	5.69	1.39	0.62	20.22	63.72	3.60
Dietary intervention	Conventional biscuit and soft drink (CB + SD)	0.57	3.75	1.75	7.82	32.57	0.02

Tested effect	Treatment	TDF (%)	FRU (%)	GLU (%)	SUC (%)	TAC (%)	TPC (%)
	Conventional biscuit and apple juice (CB + AJ)	0.70	3.75	1.75	7.82	32.57	1.13
	Reformulated biscuit and apple juice (RB + AJ)	2.26	4.10	1.88	8.37	31.10	2.42

The effect of technological intervention was assessed by administering aliquots of CB and RB dissolved in 1 mL water and providing 1.58 g/kg$_{bw}$ available carbohydrate based on their formulation (Alongi et al. 2019). The effect of dietary intervention was tested by combining CB and RB with 1 mL apple

juice, and CB with 1 mL of soft drink, standardising the total amount of available carbohydrates to 1.58 g/kg$_{bw}$ ~~body weight~~.

Blood samples were collected from tail veins at 0 (prior to the administration), 15, 30, 45, 60, 90 and 120 (after the administration) min to assay plasma glucose concentration (Accu-Chek®, Roche Diabetes Care Italy S.p.A., Monza, Italy), which was plotted against time (Belobrajdic et al. 2016). The area under curve (AUC) of glucose concentration was computed by the trapezoidal rule (Chiou 1978).

Consumer response

Conjoint analysis was used to evaluate consumer preference towards biscuits by decomposing total preference in partial preferences relevant to independent product attributes (De Pelsmaeker et al. 2017; Sillani et al. 2017). Five attributes of biscuits containing apple pomace were selected as discrete experimental variables and named “package size”, “sugar”, “fibre”, “diabetes” and “glycaemic index”. Different levels were associated with each experimental variable (Table 2).

Table 2. Experimental variables defining biscuit attributes and relevant levels used for the conjoint analysis.

Experimental variable	Level
Package size	“Single portions”; “400 g”
Sugar	Absent; “Low sugar content”; “Without sugars”
Fibre	Absent; “High fibre”; “Source of fibre”
Diabetes	Absent; “Suitable for diabetics”
Glycaemic index	Absent; “Low glycaemic index”

Experimental variables were combined according to an orthogonal experimental design, obtaining twenty product profiles, which represented the information available to consumer on a hypothetical biscuit label. A non-probabilistic sample of 300 consumers, equally distributed among men and women (age 18–73), was recruited at the University of Udine, Italy. The response of consumers towards a biscuit label reporting different information was assessed, by asking consumers to fill up a structured questionnaire, indicating, for each product profile, their preference on a 1–100 scale, without any prior information about biscuit reformulation.

Consumers were also asked to indicate their weight and height, and these data were used to compute the Quetelet’s index, i.e. the BMI (Khosla and Lowe 1967).

Statistical analysis

The required sample size was determined by carrying out *a priori* analysis, using the software GPOWER (Erdfelder et al.1996). An F test – ANOVA (fixed effects, omnibus, one-way) was applied with $\alpha = 0.05$, power = 0.80, and effect size $f = 0.84$.

Results are averages of at least three measurements carried out on two replicated samples and are reported as mean ± standard deviation. In the case of *in vivo* trials, results are averages of five measurements and are reported as mean ± standard error. The Shapiro-Wilk test was used to evaluate normality of the data, while the Bartlett procedure was used to test the homogeneity of variances. Analysis of variance (ANOVA) was performed with $p < 0.05$ and by applying the Tukey’s post hoc test. For *in vivo* trials, significance in the range $0.05 < p < 0.10$ was accepted as a trend (Thiese et al. 2016), and the Duncan’s post hoc test was carried out.

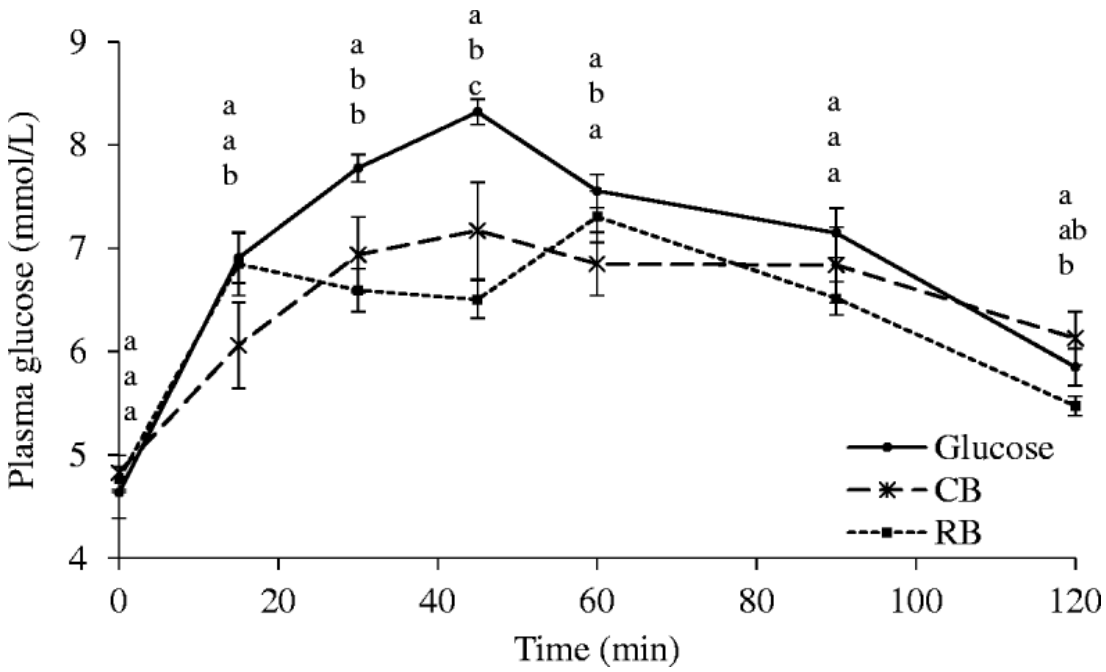
For conjoint analysis, IBM SPSS Statistics 20 (Armonk, NY, USA) was used to calculate partial preference values, their relative importance, which were reported as mean ± standard error, and the model goodness of fit (Pearson’s R and Kendall’s τ). t-Test was performed with $p < 0.05$.

Results and discussion

Effect of reformulation and dietary interventions on glycaemia

Figure 1 shows the plasma glucose levels during two hours after administration of glucose, conventional (CB) and reformulated biscuits (RB).

Figure 1. Glycaemic response in healthy rats after administration of glucose, conventional (CB) and reformulated (RB) biscuit. Equal letters (a-b) indicate no significant differences among treatments at each time.



Both CB and RB samples presented lower glycaemic curves than the control group which only received the glucose load. However, differences were observed in the glycaemic profile of CB and RB. In particular, an initial peak after 15 min from RB administration was observed. Such an initial steeper increase in plasma glucose level as compared to CB

could be attributed to the concomitant presence of fructose and sucrose. Fructose was actually found in RB (Table 1) due to its reformulation by using apple pomace as an ingredient to partially replace wheat flour (Alongi et al. 2019).

The initial faster glycaemic rise in RB as compared to CB can be thus attributed to a change in the qualitative composition of sugars, and in particular to the presence of fructose, as its absorption is known to be fastened via the disaccharidase-related transport system in the presence of other sugars, such as sucrose and glucose (Ushijima et al. 1995).

However, it is noteworthy that biscuit reformulation caused a shift in the glycaemic peak from 45 to 60 min. Such pattern change can be related to the increase in indigestible carbohydrate observed for RB. In fact, as previously reported by Alongi et al. (2019) and shown in Table S1, apple pomace contained a considerable concentration of dietary fibre (nearly 40%). As a result, RB contained nearly 6% TDF, whereas CB only contained 1.6% of it (Table 1). Interestingly, RB could be claimed as "source of fibre", as they contained ≥ 3% TDF, and nearly satisfied the "high fibre" claim, requiring TDF ≥ 6% (Reg. (EU) No 432/2012~~The European Parliament and the Council of the European Union 2006~~). AQ4

One third of TDF in AP, and thus in RB, accounted for the soluble fraction (SDF), which may delay glucose release by hurdling amylolytic cleavage through the formation of a gel able to entrap starch granules (C. S. Brennan 2005). The remaining fraction of TDF was represented by insoluble fibre (IDF), which can indirectly affect the glycaemic response by modifying the intestinal transit rate (Wilfart et al. 2007). Therefore, both SDF and IDF contributed to slowing down the digestion and absorption of carbohydrates, thus modifying the postprandial glycaemic response.

It should be highlighted that even though total available carbohydrate (TAC) content decreased from 72% in CB to 64% in RB (Table 1), due to the partial replacement of wheat flour, having 84% TAC (USDA2019), with AP, having 18% TAC (Table S1), such quantitative change did not affect the glycaemic response. In fact, the total amount of available carbohydrates was standardised for all experiments to 1.58 g/kg body weight. Still, the lower amount of total carbohydrates in reformulated biscuits could affect the glycaemic load of food, considering the same serving size (Scazzina et al. 2016).

Besides its high fibre content, apple pomace is also known to contain a considerable amount of phenolic compounds (Wolfe and Liu 2003). These are recognised to exert an anti-diabetic effect through different mechanisms, including the inhibition of carbohydrate digestion and glucose absorption, the stimulation of insulin secretion from the pancreatic β-cells, the modulation of glucose release from the liver, the activation of insulin receptors and glucose uptake in the insulin-sensitive tissues, and the modulation of intracellular signalling pathways and gene expression (Hanhineva et al. 2010). In this regard, Table 3 reports the phenolic profile of apple pomace, as well as of the conventional and reformulated biscuits. As expected, the phenolic content in CB was negligible, while that in the reformulated biscuit accounted for about 36 μg/g. Interestingly, such a phenolic content was consistent with that expected in RB biscuit based on apple pomace content in the dough (10.3% w/w). Despite the thermal sensitivity of some phenolic compounds, processing, i.e. baking, did not affect their concentration. This could be attributed to a protective

effect of the matrix. Actually, this was also previously observed for apple juice, in which, the thermal treatment (i.e. pasteurization) caused an increase of the phenolic concentration (De Paepe et al. 2014; Alongi et al. 2018).

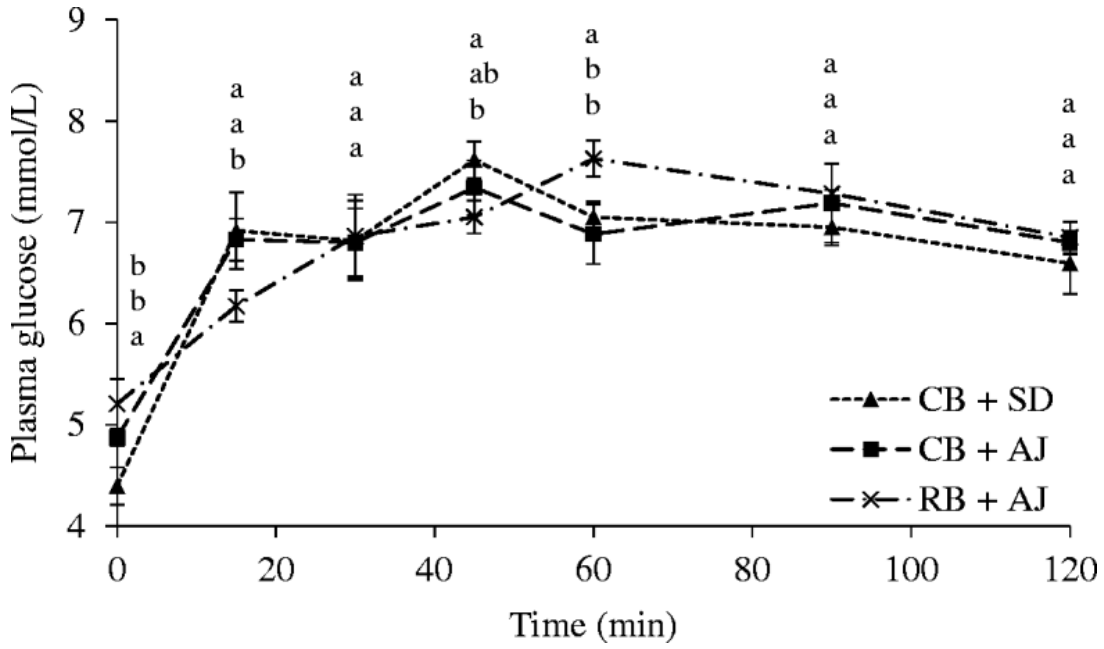
Table 3. Pphenolic profile of Aapple juice (AJ), apple pomace (AP), conventional (CB) and reformulated (RB) biscuits.

Compound	AJ (µg/mL)	AP (µg/g)	CB (µg/g)	RB (µg/g)
Chlorogenic acid	8.52 ± 0.52	25.45 ± 1.54	0.046 ± 0.001	5.67 ± 0.33
p-Coumaroylquinic acid	2.48 ± 0.12	2.86 ± 0.01	n.d.	0.34 ± 0.03
Hydroxycinnamic acids	11.00 ± 0.64	28.31 ± 1.55	0.046 ± 0.001	6.00 ± 0.30
Phloretin Xyloglucoside	0.933 ± 0.041	20.04 ± 0.60	0.287 ± 0.013	3.96 ± 0.35
Phloridzin	2.250 ± 0.001	75.38 ± 2.80	0.108 ± 0.005	9.83 ± 0.56
Dihydrochalcone derivatives	3.183 ± 0.042	95.42 ± 3.40	0.395 ± 0.018	13.79 ± 0.91
Procyanidin B2	0.534 ± 0.005	25.73 ± 1.62	n.d.	0.93 ± 0.03
(−) Epicatechin	0.159 ± 0.002	26.66 ± 0.76	n.d.	2.30 ± 0.05
(+) Catechin	0.735 ± 0.035	17.59 ± 0.68	n.d.	1.22 ± 0.07
Flavan-3-ols	1.427 ± 0.032	69.98 ± 1.55	n.d.	4.46 ± 0.01
Quercitin-3-*O*-galactoside	0.891 ± 0.009	46.92 ± 2.78	0.045 ± 0.004	6.99 ± 0.52
Quercitin-3-*O*-xyloside	0.077 ± 0.002	7.62 ± 0.05	n.d.	0.84 ± 0.07
Quercitin-3-*O*-rhamnoside	0.372 ± 0.004	18.75 ± 0.81	n.d.	2.57 ± 0.10
Quercitin-3-*O*-arabinoside	0.012 ± 0.001	1.20 ± 0.05	n.d.	0.20 ± 0.02
Others quercitin-pentoside	0.070 ± 0.001	11.54 ± 0.10	n.d.	1.18 ± 0.05
Flavonols	1.42 ± 0.01	86.02 ± 3.59	0.045 ± 0.001	11.78 ± 0.65
Total phenolic compounds	17.03 ± 0.70	279.72 ± 6.99	0.486 ± 0.001	36.03 ± 1.85

n.d.: not detected.

Dietary interventions were also tested by administering conventional (CB) and reformulated biscuits (RB) together with different beverages, which were represented by apple juice (AJ) and a soft drink (SD) mimicking the juice sugar composition (Table S1), i.e. containing 57.3 mg/mL fructose, 26.3 mg/mL glucose and 12.6 mg/mL sucrose. Figure 2 shows the plasma glucose level during two hours after the administration of the different treatments reported in Table 1.

Figure 2. Glycaemic response in healthy rats after administration of conventional biscuit (CB) + soft drink (SD) or apple juice (AJ), and reformulated biscuit (RB) + AJ. Equal letters (a-b) indicate no significant differences among treatments at each time.



After 15 min from the ingestion of CB with AJ or SD, a peak of glycaemia appeared as compared to CB alone (Figure 1) due to the presence of fructose in the beverages (Ushijima et al. 1995). Although a similarity in the glycaemic patterns was observed when CB was ingested with the beverages, a slightly flatter curve was found for the CB ingested with AJ as compared to its consumption with the SD. In fact, the glycaemic peak at 45 min was less pronounced in the case of CB administered with AJ, while an increase in glycaemia was observed at 90 min. These differences may be attributed to a matrix effect; differently from SD, besides sugars, AJ also contained other molecules, such as dietary fibre, even if in a small amount (0.2%), and phenolic compounds (Table 3). The phenolic content of commercial apple juice indicated that chlorogenic acid (50%) was the main compound, followed by p-coumaroylquinic acid (15%) and phloridzin (13%). Such composition was comparable to what previously observed by other authors (Gerard and Roberts 2004; Alongi et al. 2018), which demonstrated the efficacy of apple juice in inhibiting α-glucosidase, considering ordinarily consumed amounts of juice.

In the light of the overall positive outcome derived from both the ingestion of CB with AJ (Figure 2) and the reformulation intervention (Figure 1), these effects were studied conjointly by administering RB with AJ (Figure 2). The glycaemic peak shift from 45 min to 60 min observed upon reformulation was maintained also when RB was consumed with AJ. By comparing the plasma glucose pattern of RB and RB + AJ (Figures 1 and 2), it can be noticed that the glycaemic response was only slightly affected by the presence of the juice, while reformulation played the major role. These results can be attributed to the lower abundance of phenolic compounds in the juice than in AP-containing biscuit (Table 3).

Table 4 shows the area under curve (AUC) relevant to glucose, and reformulation and dietary treatments, which were computed from the plasma glucose response graphs (Figures 1 and 2). CB and RB interventions induced a decrease in AUC as compared to the administration of glucose, whereas no differences were observed between CB and RB AUC values. Moreover, all the dietary treatments did not differ among each other, but AUC was lower as compared to that of glucose. Still, the administration of AJ, instead of SD, with CB did not modify AUC. This remained unchanged also when AJ was administered together with RB, instead of CB. Although the different interventions did not produce significant changes in terms of AUC, it should be kept in mind that glucose tolerance information of physiological or clinical interest may be lost by only using a simple summary measure, such as AUC. On the contrary, this information can be preserved by considering the glucose curve shape (Frøslie et al. 2013), which actually showed differences depending on the administrated treatment (Figures 1 and 2). In fact, M. A. Brennan et al. (2012) did not find differences in the AUC upon the administration of an oat-bran containing snack as

compared to the control sample, but observed a lower and prolonged glucose release due to the presence of oat fibre, suggesting an effect on the glycaemic response and thus on satiety.

Table 4. Area under curve (AUC) of the plasma glucose responses to glucose, conventional (CB) or reformulated (RB) biscuit, CB + soft drink (SD) or + apple juice (AJ), and RB + AJ. Equal lowercase letters (a) indicate no significant differences among treatments within tested effect. Equal uppercase letters (A–C) indicate no significant difference among all treatments.

Tested effect	Treatment	AUC (mmol/L/min)
	Glucose	852 ± 9A
Reformulation intervention	CB	790 ± 24a,C
	RB	777 ± 8a,C
Dietary intervention	CB + SD	819 ± 8a,B
	CB + AJ	830 ± 19a,B
	RB + SD	833 ± 9a,B

Overall, these results confirmed the positive effect of apple pomace as a functional ingredient in reducing the glycaemic response, previously demonstrated by in vitro studies (Alongi et al. 2019). Thus, a reformulated food containing apple pomace could be suggested for tackling type 2 diabetes.

Effect of reformulation on consumer response

A critical issue determining the effective delivery on the market of a reformulated biscuit is represented by the labelled information, which indeed affects the reaction of consumers towards their consumption. In fact, the sensory acceptability was previously studied and overall positive sensory outcomes were assessed (Alongi et al. 2019). Conjoint analysis was thus applied to assess consumers' responses towards a biscuit label reporting different information. Claims associated with the content of compounds potentially affecting type 2 diabetes, as well as claims more directly referring to the disease, were considered (Table 2). Partial preference coefficients and relative importance of each label information in defining consumer response are reported in Table 5.

Table 5. Partial preference coefficients and relative importance of different label information in defining consumer preference. Pearson's $R = 0.98$ ($p < 0.0001$); Kendall's $\tau = 0.85$ ($p < 0.0001$); Kendall's τ for controls = 1.00 ($p < 0.05$).

Information	Partial preference coefficient	Relative importance
400 g	1.16 ± 0.33	15.03 ± 0.86
Single portions	−1.16 ± 0.33	
Suitable for diabetics	−2.38 ± 0.33	17.41 ± 0.71
Absent	2.38 ± 0.33	
Low glycaemic index	0.11 ± 0.33	14.81 ± 0.63
Absent	−0.11 ± 0.33	
Low sugar	4.03 ± 0.44	27.58 ± 0.78
Sugar-free	−0.98 ± 0.51	
Absent	−3.05 ± 0.51	
Source of fibre	1.13 ± 0.44	25.18 ± 0.80
High fibre	2.75 ± 0.51	
Absent	−3.88 ± 0.51	

The partial preference coefficient presented positive or negative values, the former indicating a higher preference as compared to the latter. Information about the package size was provided, since controlling the portions represents a key feature to manage diet-related diseases, such as diabetes (Pedersen et al. 2007). Surveyed consumers preferred the 400 g instead of single portions. Interestingly, consumers preferred not to be provided with information directly referring to diabetes ("suitable for diabetics"), whereas the information relevant to the glycaemic index did not affect consumer response in a significant manner. Considering sugar content, which indirectly refers to diabetes as its intake negatively affects diabetes, consumers preferred the "low sugar" to the "sugar-free" claim, indicating a sugar content below 5%, and 0.5%, respectively (Reg. No 1924/2006). The lower preference evidenced in the case of the absence of sugar could lie in the less natural perception of the product by consumers (Siegrist and Sütterlin 2017). In fact, short dough biscuits without sugars generally present sweeteners in their formulation, which might be negatively perceived by consumers. In fact, these usually look for a compromise between health and naturalness perception, as well as taste, which is the crucial feature affecting food choices (Kaur and Das 2011; Giordano et al. 2018). On the contrary, considering the fibre, which is known to positively affect glycaemic response and was contained in high concentration in apple pomace, consumers preferred the "high fibre" over the "source of fibre" claim

referring to a concentration ≥ 6% and ≥ 3%, respectively (Reg. No 1924/2006). Interestingly, it can be noticed that on the one hand consumers preferred not to be provided with information directly referring to the disease, but on the other hand, they expressed a higher preference when information about nutritional features affecting the disease was provided. In this regard, it should be kept in mind that consumers generally prefer information highlighting the health-promoting effect in a general manner despite directly relating to a specific disease (Schnettler et al. 2019). In fact, consumers generally do not dispose of the knowledge required to understand specific and technical information and to relate it to a beneficial effect on their own health (Bech-Larsen and Scholderer 2007).

Table 5 also shows the relative importance of each attribute, indicating the "weight" given by consumers during the expression of their preference. Information relevant to sugar content was most influencing, followed by fibre content. The absence of indication about the disease played a crucial role in determining consumer preference as well. On the contrary, lower importance was attributed to the glycaemic index, as well as to the package size.

In addition, partial preference and relative importance of each information were compared among BMI classes. It should be kept in mind that the consumers involved in the survey were not overt diabetics. Still, nearly 20% of the interviewed consumers were overweight or even obese, according to their BMI class (

WHO 2019). Overall, no differences were observed among BMI classes, indicating that the information provided equally affected all the population, which in general preferred learning about the nutritional features instead of the disease, in agreement with literature findings (Van Kleef et al. 2005; Bimbo et al. 2017). This means that marketing campaigns promoting functional foods by highlighting the content of bioactive compounds and the overall effect on wellbeing can play a role not only in the management of diet-related diseases but also towards the maintenance of a healthy status. As obesity represents one of the major risk factors for type 2 diabetes, further research is required involving overt diabetics under treatment, to understand how consumer response changes between diseased subjects and those healthy or unaware of their disease.

Conclusions

The present research highlighted that the reformulation of bakery products by using ingredients rich in fibres and other bioactive compounds might be a valuable option to reduce the glycaemic impact of these foods, thus representing a reliable dietary tool to manage and even prevent type 2 diabetes. In addition, acquired results demonstrated the possibility to valorise by-products derived from vegetable processing, e.g. apple pomace, which are still rich in dietary fibre and bioactive compounds, such as phenolic ones. Reusing them as functional ingredients would contribute to increasing the sustainability of the food production system.

To our knowledge, this is the first attempt to investigate in depth the combination of technological intervention, i.e. reformulation, with dietary intervention towards the prevention of type 2 diabetes. Such approach could be extended to the study of food-drug combination, to identify synergistic behaviours that may allow drug dosage reduction. Still, acquired results need to be validated in humans. In addition, there is a need for a deeper understanding of the mechanisms behind the observed functionality to steer food design with an *a priori* knowledge.

Results also pointed out that the choice of information provided to consumers plays a critical part in determining the success of functional foods on the market. Choosing a nutritional claim must thus consider not only the target population, such as diabetics and obese subjects in this case but also healthy persons consuming functional foods to maintain their wellbeing.

Disclosure statement

The authors report no conflict of interest.

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