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INVITRO GLYCEMIC RESPONSE OF GLUTEN-FREE BISCUITS AND THEIR MAIN INGREDIENTS: A SYSTEMATIC REVIEW

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Running title
Invitro GI of gluten-free biscuits

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Abstract

Gluten-free (GF) biscuit uses different ingredients for substituting gluten-based ingredients which gives rise to varied glycemic index (GI) based on the nature of the ingredient. Invitro starch digestion is a non-invasive laboratory method to estimate starch digestion and GI. This study aimed at performing a systematic review on invitro starch digestion conducted in GF biscuits. The electronic search using PRISMA guidelines was conducted in PubMed, DOAJ and Google Scholar resulting in 18 eligible studies. All of the nine finally selected studies showed medium risk of bias evaluated using the QUIN tool. The number of studies that reported high GI were 1; medium–6 and low–2 in the experimental biscuits. All studies that reported starch hydrolysis revealed a higher dietary fibre or slowly digestible starch content. All selected studies reported improved nutritional profile, variable hardness and overall acceptability based on the choice and processing method of ingredients. This systematic review identified several gaps such as the need to study nature and processing techniques of ingredients, optimizing proportion of ingredients to improve palatability and consumer acceptability of biscuits. Further studies are required to understand the impact of several GF ingredients on the starch digestion and glycemic index of biscuits.

Introduction

Biscuits are one of the highly consumed snacks world-wide and the dominant food product in the category of fast-moving consumer goods. It is produced in extensive variety of flavours, has a long shelf life and a high level of consumer acceptance. However, the gluten content of biscuits is a risk factor to public health considering the increase of gluten sensitivity among the population (Goubgou et al., 2021). The prevalence of celiac disease is estimated to be 1 to 1.4% worldwide and the primary treatment in maintaining the symptoms is the gluten-free (GF) diet (Poslt Königová et al., 2023). Furthermore, gluten peptides may result in enteropathy, insulin resistance, and leptin resistance, all of which may have negative impact on obesity and Type 1 and Type 2 diabetes (Haupt-Jorgensen et al., 2018). Owing to the recent trend in consumer preferences and health awareness, GF food products are raising global interests with the usage of ingredients such as pseudocereals, millets and pulses to replace the gluten from wheat, barley, rye and oats (Adiamo et al., 2018).

Glycemic index (GI) is a primary metric for evaluating the therapeutic potential of biscuits in public health, given their status as widely consumed convenience foods (Di Cairano et al., 2018). The GI of biscuits depends on various factors like choice of the ingredients, their quality, and food processing techniques (El Khoury et al., 2018). The recent trends indicate that different ingredients are being explored to improve the nutritional quality, texture and consumer acceptability of GF biscuits (Melini & Melini, 2019). Different processing methods of ingredients such as fermentation and germination are being researched. Hydrocolloids, sugar substitutes, resistant starches and functional ingredients such as plant by-products are also added to the biscuit formulations to achieve desirable GI and improved quality (Di Cairano et al., 2018).

To estimate the GI of food products, the World Health Organization (WHO) has recommended invivo method which is an invasive process involving drawing blood samples periodically from the participants several times. Owing to the time, cost and labour, invitro starch digestion and calculated glycemic index are preferred, which is a non-invasive laboratory procedure (Magaletta et al., 2010). Invitro starch digestion method has gained significance as it could provide insights on the nature of ingredients and the effect of different processing methods before conducting invivo analysis or large-scale population trials (Sulung et al., 2023).

Therefore, this systematic review aimed at identifying the invitro starch digestibility of the different gluten-free ingredients used in the preparation of biscuits to understand the properties of ingredients which will be helpful in future preparations of gluten-free biscuit formulations.

Materials and Methods

To ensure consistency, our systematic review followed PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines for reporting. The review aimed to identify studies investigating the invitro starch digestibility of gluten-free biscuits.

Search strategy

The PICOT framework was used to define the research question and identify appropriate key words (***Supplementary Table 1***). An electronic search was carried out in three databases – PubMed, Directory of Open Access Journals (DOAJ) and Google Scholar to identify studies regarding invitro glycemic response and gluten-free biscuits. The articles were searched using the keywords, "glycemic index", "invitro" and "gluten-free biscuits". The detailed search strategy and keywords for each database is given in ***Supplementary Table 2***. Filters such as articles published in the last 10 years and English language were applied in PubMed while the first 200 articles were selected from Google Scholar as recommended by Bramer et al. (Bramer et al., 2017).

Eligibility criteria

The studies were included in this systematic review if they met the following inclusion criteria. Studies with invitro starch digestion as the primary or secondary outcome conducted in gluten-free biscuits were selected. The articles published only in English language were included. Studies published between 2016 and 2026 (last 10 years) chosen. Full text original research papers were included. Review articles, editorials and articles written in other languages were excluded.

Data Extraction

Data extraction was done manually using standard structure and managed using Excel software. The citations were managed using Zotero Reference Management software. Information such as author, year of publication, place of study, control biscuit, experimental biscuit, invitro glycemic response/starch hydrolysis related outcomes and other outcomes.

Quality Assessment

The Quality Assessment Tool for In Vitro Studies (QUIN tool) as used for assessing the quality and risk of bias of the selected articles (Sheth et al., 2024). The QUIN tool considers twelve criteria such as clearly stated aims/objectives, detailed explanation of sample size calculation, detailed explanation of sampling technique, details of comparison group, detailed explanation of methodology, operator details, randomization, method of measurement of outcome, outcome assessor details, blinding, statistical analysis and presentation of results. Each criterion is scored as 0 (not specified), 1 (not adequately specified), 2 (adequately specified) or NA (not applicable). The final score is calculated as (Total score×100)/(2×number of criteria applicable). Based on these scores, the studies are categorized as high risk (<50%), medium risk (50% to 70%), or low risk (>70%).

Result and Discussion

Characteristics and Quality of selected studies

The literature search yielded a total of 210 articles out of which 18 were chosen upon screening the title and abstract. A total of 9 articles were included in this systematic review after removing duplicates and applying the inclusion and exclusion criteria. The PRISMA flow chart of selection process is given in ***Fig 1***.

Risk of bias assessment using the QUIN tool revealed that all the included studies were rated as having a medium risk of bias, with scores ranging from 50% to 70% (***Table 1***). Most of the selected studies were conducted in Italy (n=5), followed by Portugal (n=1), Nigeria (n=1). Place of study was not mention in two studies. Different types of wheat flour were used for the preparation of control biscuits in four studies while the remaining studies used study-specific gluten flours as controls. The details of the study and their outcomes are presented in ***Table 2***.

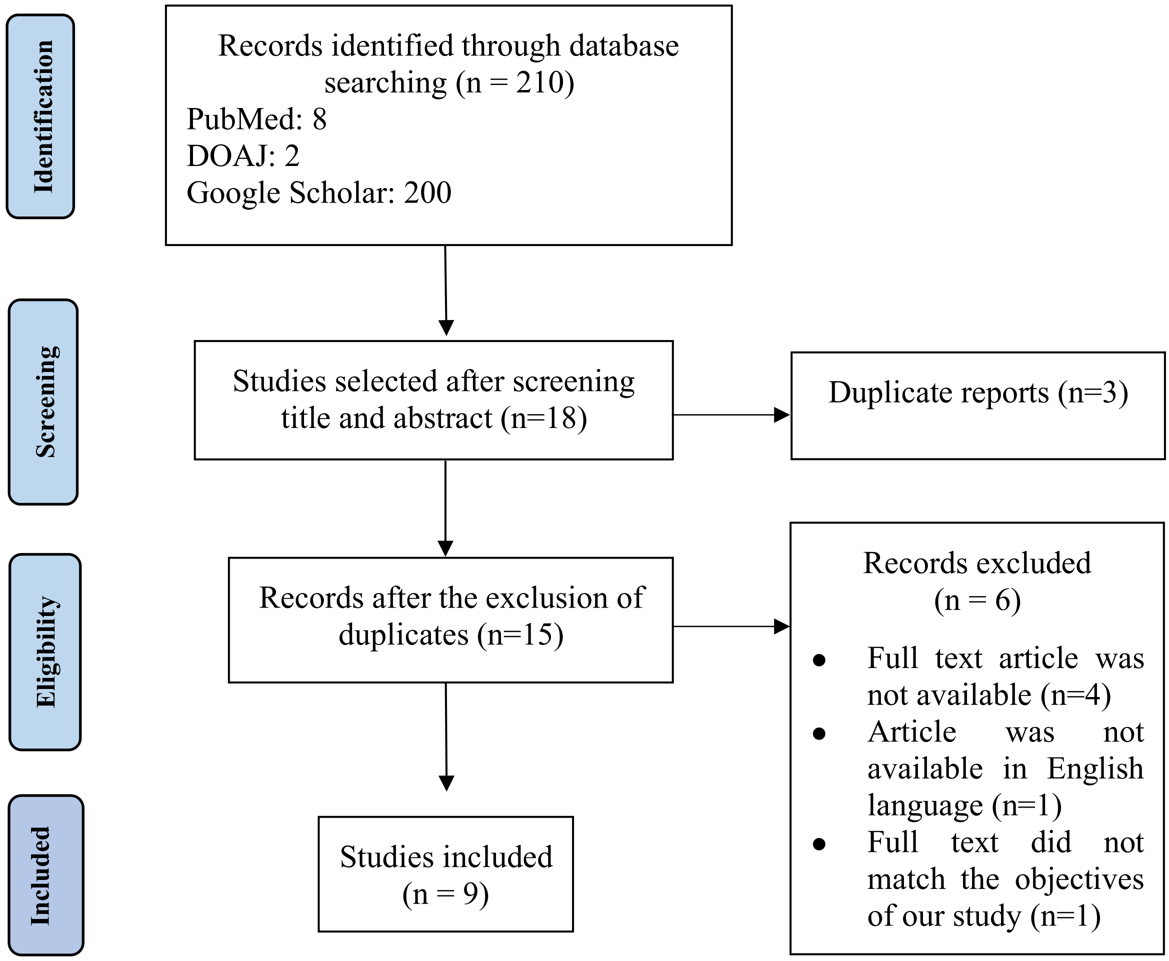

Fig 1. PRISMA Search strategy for selection of articles

Invitro glycemic response of gluten-free biscuits and their ingredients

Out of 9 studies, 5 studies reported calculated GI indicated that addition of gluten flours decreased the glycemic index (GI) of the biscuits. Two studies used wheat flour and corn flour

for preparation of control biscuits, which were reported to be high GI, >70 (Di Cairano et al., 2021; Ozgolet et al., 2025). Millet and specific rice varieties with higher amylose and resistant starch content resulted in medium GI. Ariete long A grain and Type III Basmati grain varieties used as control biscuits were medium GI (≈66), but replacing milled rice flours with brown rice flours of the same rice varieties did not show much difference in the GI values and the products remained in 57-60 GI range (Pereira et al., 2025). Usage of millet such as germinated finger millet control biscuits also showed medium GI (58). Increasing substitution of germinated finger millet with germinated Bambara groundnut flour resulted in gradual reduction of GI with 51.33 (low GI) for 40% replacement (Chinma et al., 2022).

Another study reported that whole millet reduced the GI of the biscuits in combination with pseudocereal and pulses (low GI) than sorghum based biscuits in similar combination (medium GI) (Di Cairano et al., 2021). However, addition of peeled and unpeeled sweet potato flour did not reduce the GI of the biscuits (> 85) possibly because of the baking process increasing the GI of sweet potatoes (Ozgolet et al., 2025). One study registered that using sucrose replacers such as inulin and maltitol reduced the GI of the biscuits from with even 30% replacement and gradually decreasing GI from high to medium category with increasing sucrose replacers proportion (Di Cairano et al., 2022). The starch digestion rate of foods is directly associated with higher slowly available glucose and lower rapidly available glucose content (Rojas-Bonzi et al., 2020). This is undisputedly evident in the results of all selected studies in this systematic review.

Table 1. Risk of bias assessment in the selected studies

Author and Year	Clearly Stated Aim/Objectives	Detailed Explanation of Sample Size Calculation	Detailed Explanation of Sampling Technique	Details of Comparison Group	Detailed Methodology	Operator Details	Randomization	Methods of Measurement of Outcome	Outcome Assessor details	Blinding	Statistical analysis	Presentation of results	Total Score	Final Score/ Risk of bias
Pereira et al., 2025	2	0	0	2	2	0	NA	2	0	0	2	2	12	54.55/ Medium
Ozgolet et al., 2025	2	0	0	2	2	0	NA	2	1	0	2	2	13	59.09/ Medium
Yu et al., 2022	2	0	1	1	2	1	NA	2	0	0	2	2	13	59.09/ Medium
Chinma et al., 2022	2	0	2	0	2	0	NA	2	0	0	2	2	12	54.55/ Medium
Di Cairano et al., 2022	2	0	0	2	2	1	NA	2	0	0	2	2	13	59.09/ Medium
Di Cairano et al., 2021	2	0	1	2	2	0	NA	2	0	0	2	2	13	59.09/ Medium
Cervini et al., 2021	2	0	2	2	2	1	NA	2	0	0	2	2	15	68.18/ Medium
Giuberti et al., 2021a	2	0	2	2	2	0	NA	2	0	0	2	2	14	63.64/ Medium
Giuberti et al., 2021b	2	0	0	2	2	0	NA	2	0	0	2	2	12	54.55/ Medium

Five studies reported the starch hydrolysis index of the biscuits after invitro starch digestion. All studies uniformly reported higher HI for control biscuits and gradual decrease in HI upon increasing the content of functional ingredients. Amylose, protein, resistant starch and the usage of sugar alcohols were involved in the reduction of GI in the selected studies. Resistant starch type 2 (RS2), which is formed from the high amylose content, delays starch digestion and reduces postprandial glucose response by resisting enzymatic hydrolysis (Harris, 2019). Proteins surround and structurally encapsulate the starch molecules which inhibits its access to enzymatic hydrolysis by α-amylase and thus reduces digestibility (Zhang et al., 2025). Similarly, sugar alcohols having a low digestibility reduces the glycemic index of foods which is proven in several invitro and invivo studies (Grembecka, 2015). Such synergistic effects of non-starch components in biscuits greatly affect the microstructure of the biscuits during baking affecting the starch digestibility.

Effect of the ingredients on other nutritional, textural and sensory parameters of gluten-free biscuits

Nutritional parameters were enhanced in the experimental biscuits. Depending on the type of ingredient, certain nutritional parameters increased in the biscuits evidently, e.g. addition of millets resulted in increased mineral content and addition of pulses increased the protein content. Addition of carbohydrate rich ingredients like amylose starch, sweet potato flour and resistant dextrin significantly reduced the protein content in the selected studies. Literature shows that rice, tapioca, potato, corn and simple starch-based ingredients are inferior to pseudocereal and leguminous flours in terms of nutrition. Usage of buckwheat, amaranth, quinoa, millets, soy, egg, etc., improve the phenolic content and antioxidant activity with a reduced antinutrient content (El Khoury et al., 2018).

Di Cairano et al., (2021) reported no significant difference in the breaking point was observed between whole millet and sorghum. Resistant starch and moringa leaf powder increased the hardness of the biscuits leading to poor texture than control biscuits. High fibre increases the water binding capacity and reduces the gas retention capacity of the dough which causes hardness in the biscuits (Punfujinda et al., 2025). However, Pereira et al., reported that inclusion of rice bran significantly decreased the hardness contrary to existing literature (Ayoub et al., 2022). Addition of chickpea flour resulted in higher hardness while germinated Bambara groundnut flour reduced the hardness. Increased protein weakens the rheological properties of dough resulting in hardness (Nogueira & Steel, 2018). However, processing methods such as germination and fermentation causes modifications in the protein structures resulting in decreased hardness of biscuits (Di Cairano et al., 2018).

Out of the five studies that reported sensory analysis, 3 studies reported no significant differences while two studies reported significant differences among the formulations. One study reported that sorghum tasted better than millets. Beany/legume/nuts flavour was reported by all two studies that used legumes and one study that used resistant starch and sugar alcohols. All selected studies reported that their formulations achieved overall acceptance though bitter taste and beany flavour were attributed to high polyphenol and oligosaccharide contents respectively. Inclusion of GF ingredients have reported mixed results in biscuits such as taste- bland, bitter or sweet; colour- dark; aftertaste- strong or bitter; flavour- intense or inferior; mouthfeel- crispy, crumbly or nutty, which often lead to poor to moderate overall acceptability of the GF biscuits. Little importance is given to sensory attributes during the formulation of GF biscuits (Di Cairano et al., 2018; El Khoury et al., 2018). This systematic review is also in congruence with the idea that sensory attributes were customarily secondary outcomes in many of the selected studies but require more focussed attention in future studies.

Conclusion

The current systematic review has revealed that the calculated glycemic index of GF biscuits in six studies was in the medium category (56-69) owing to the presence of amylose, resistant starch, proteins and sugar alcohols. The nutritional quality of GF seemed to be enhanced; however, the textural and sensory properties such as taste and hardness of the biscuits were compromised in all studies leading to poor to medium acceptability. A key consideration is that biscuit formulation requires optimization of glycemic index, nutritional and textural parameters simultaneously working on sensory characteristics to translate findings from lab to market. There is a need to study the effect of nature of ingredients when undergoing baking and various processing methods of ingredients on the GI of biscuits. Similarly, the usage of hydrocolloids, gums, sugar alcohols and their relation to starch digestion in biscuits are under-explored in research. More robust evidence is required on invitro and invivo mechanisms to comprehend the impact of several GF ingredients on the starch digestion and glycemic index of biscuits. Current literature on GF biscuits reveals a huge lacuna in various areas and need to studied in depth to achieve a healthier and palatable alternative to gluten biscuits.

Table 2. Data extraction of selected studies

Author	Place of Study	Control Biscuit	Experimental biscuit	Invitro starch digestion related outcomes	Other outcomes
Pereira et al., 2025	Portugal	Biscuits made with 100% rice milled flour from the Ariete long A grain (A0) and Type III Basmati grain varieties (B0)	A1: 50% Ariete milled flour with 50% rice bran; A2: 75% Ariete brown flour with 25% rice bran; B1: 50% Type III milled flour + 50% rice bran; B2: 75% Type III brown flour with 25% rice bran.	The lowest GI was found for 50% TMF + 50% RB (57.06 ± 0.59), followed by 50% AMF + 50% RB (60.31 ± 0.32) and 75% TBF + 25% RB (60.59 ± 0.10) ($p<0.05$)	The rapidly digestible starch was the least for 50% TMF + 50% RB while the slowly digestible starch was the highest for 100% TMF (control). Type III Basmati grain recommended for reducing GI of the products.
Ozgolet et al., 2025	Not specified	Wheat based biscuits and corn starch–based biscuits	PSPB100: biscuits made from peeled sweet potato flour; PSPB50: biscuits made from peeled sweet potato flour blended with corn starch (50%–50%); USPB100: biscuits made from unpeeled sweet potato flour; USPB50: biscuits made from unpeeled sweet potato flour blended with corn starch (50%–50%).	All control biscuits showed GI above 90 and experimental biscuits showed GI above 85.	The experimental biscuits showed significant increase in potassium, magnesium, calcium, iron and polyphenol contents.
Yu et al., 2022	Not specified	100% wheat flour (WF)	Single substitution by 60% of Common buckwheat starch (ST); single substitution by Resistant dextrin (RD) to achieve 5g dietary fibre /100 g biscuits; Double substitution by 60% of ST and RD to achieve 5g dietary fibre /100 g biscuits	Single substitution by RD and double substitution by ST and RDT had the least digestion rate coefficient (*k*) and the highest total undigested starch ($C\infty$) ($p<0.05$)	The invivo GI analysis of the biscuits with the double substitution by ST and RD was 47. Single substitution with RD increased the firmness of the biscuit while double substitution with ST and RD improved the texture of biscuits.
Chinma et al., 2022	Nigeria	100% Germinated Finger Millet	Germinated Finger Millet and germinated Bambara groundnut flour in the ratios 90:10, 80:20, 70:30 and 60:40	HI reduced from 33.68 ± 0.11 in 100:0 to 21.17±0.10 in 60:40. Similarly, eGI reduced from 58.20 ± 0.15 to 51.33 ± 0.19 respectively ($p<0.05$)	Increase in the proportion of germinated Bambara groundnut flour resulted in higher protein, resistant starch, slowly digestible starch, invitro protein digestibility. It also increased the total phenolic content, FRAP and ABTS activities but showed decreased overall acceptability. 80-20 proportion was recommended.

Author	Place of Study	Control Biscuit	Experimental biscuit	Invitro starch digestion related outcomes	Other outcomes
Di Cairano et al., 2022	Italy	100% sucrose as sweetener and no resistant starch	RSIN30: biscuits with resistant starch and 30% sucrose replacement by inulin; RS-M50: biscuits with resistant starch and 50% sucrose replacement by maltitol; RS-M100: biscuits with resistant starch and total sucrose replacement by maltitol	The use of sucrose replacers contributed to the reduction of in vitro glucose release and consequently of pGI with values decreasing from 84 (control) to 67 (RS-M100) ($p < 0.05$).	The browning index was the highest for control and the lowest for RS-M100. The biscuits did not show significant textural differences.
Di Cairano et al., 2021	Italy	100% soft wheat flour	1A: buckwheat to millet (50:50); 1B: buckwheat to millet to chickpea (50:30:20); 1C: buckwheat to millet to lentil (50:30:20); 2A: buckwheat to sorghum (50:50); 2B: buckwheat to sorghum to chickpea (50:30:20); 2C: buckwheat to sorghum to lentil (50:30:20)	Control biscuits had the highest HI and pGI while all these parameters were lower in 1A, 1B and 1C. The pGI for control was 70.97 ± 0.95; 1A: 56.39 ± 0.73; 1B:55.52 ± 0.23; and 1C:55.07 ± 2.31 ($p < 0.05$)	Control biscuits had the highest total starch, non-resistant starch and the lowest water absorption capacity and bulk density.
Cervini et al., 2021	Italy	Commercial all-purpose gluten flour mix with the ingredients rice flour, potato starch, sugar, hydroxypropyl-methyl-cellulose, locust bean gum, salt, mono- and diglycerides of fatty acids	Substitution of gluten flour mix with annealed sorghum starch in 0, 15, 30 and 45 g/100 g total flour basis	HI for control: 88.3 ± 2.23; 15%: 76.4 ± 1.64; 30%: 67.3 ± 1.78; 45%: 59.5 ± 1.96 ($p<0.05$).	Higher dietary fibre, resistant starch, lightness, hardness and lower protein, total starch and acceptability were found in experimental biscuits.
Giuberti et al., 2021a	Italy	Commercial all-purpose gluten flour mix with the ingredients rice flour, potato starch, sugar, hydroxypropyl-methyl-cellulose, locust bean gum, salt, mono- and diglycerides of fatty acids	Substitution of gluten flour mix with 0, 5, 10 and 15% (w/w) of *Moringa oleifera* L. leaves powder	HI for control: 81.3; 5%: 76.0; 10%: 72.0; 15%:69.3 ($p<0.05$). For gluten flour biscuits, a 10% w/w substitution might be a reasonable balance between physicochemical and nutritional qualities	Higher dietary fibre, protein and fat content while lower total starch content, starch swelling power, retrogradation tendency and textural properties in experimental biscuits.
Giuberti et al., 2021b	Italy	Commercial white wheat flour (amylose content of 28 g/100g total dry starch)	Substituting 25% and 50% w/w of common white wheat flour with high amylose starch flour from corn (amylose content: 65 g/100g total dry starch)	HI for control: 97 ± 0.7; 25% substitution: 87 ± 2.1; 50% substitution: 74 ± 1.4 ($p<0.05$)	50% substitution showed significantly lesser intake of the following meal. However, no differences were found in the feelings of fullness, satiety, hunger and hunger variation.

GI – Glycemic Index; pGI - Predicted Glycemic Index; eGI - Estimated Glycemic Index; HI – Starch Hydrolysis Index; g – grams; w/w - weight-by-weight

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Supplementary Tables

Supplementary Table 1. PICOT table

Population/Problem	In vitro starch digestion
Intervention/exposure	Glycemic response of formulated biscuits
Comparison/control	Glycemic response of whole wheat biscuit or refined wheat flour biscuit or control biscuits
Outcome measure(s)	Glycemic Index/Starch Hydrolysis Index
Time/Types of Studies included	Original research articles

Supplementary Table 2. Search Strategy

Database	**Search**
PubMed	**((gluten-free) AND (biscuit*)) AND (in vitro)** (("diet, gluten free"[MeSH Terms] OR ("diet"[All Fields] AND "gluten free"[All Fields]) OR "gluten free diet"[All Fields] OR ("gluten"[All Fields] AND "free"[All Fields]) OR "gluten free"[All Fields]) AND "biscuit*"[All Fields] AND ("in vitro techniques"[MeSH Terms] OR ("vitro"[All Fields] AND "techniques"[All Fields]) OR "in vitro techniques"[All Fields] OR "vitro"[All Fields] OR "in vitro"[All Fields])) AND (y_10[Filter])
DOAJ	**“Gluten free biscuits” AND “Glycemic Index” OR (“Starch Digestibility”) AND “In Vitro”**
Google Scholar	**“Gluten free biscuits” AND “Glycemic Index” AND “In Vitro”**

Legend

Fig 1. PRISMA Search strategy for selection of articles
Table 1. Risk of bias assessment in the selected studies
Table 2. Data extraction of selected studies