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Development of functional nougat without diabetic effects *in vivo*

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Abstract

Nougat is a confectionery foodstuff made of whipped egg white, sugar, honey, and nuts. In a traditional Italian nougat recipe, the mixture is flavoured with vanilla extract, and packed with toasted almonds. Traditional nougat is banned from the diets of diabetics due to high amount of sugar. In the present work, we aimed to develop innovative sugar-free nougat, and compare its textural and sensory properties with those of commercial nougat. Finally, we tested the residual hyperglycaemic effect in non-obese diabetic (NOD) mice, a model of type 1 diabetes. We developed a sugar-free nougat recipe by mixing xanthan gum, erythritol, and inulin with egg white and almonds using conventional industrial instruments. Technical analysis indicated that the structure, in terms of shear force, was comparable with that of traditional chewy nougat. Sensory analysis indicated that flavour and sweetness were preserved, whereas cohesiveness and fracturability changed significantly. Interestingly, the innovative food composition positively influenced two other texture parameters; solubility and adhesiveness. *In vivo* experiments showed that the number of mice in the group that was fed with experimental nougat that did not develop diabetes was significantly higher than that in the group fed with commercial nougat (66.6% *vs*. 33.0%; $p < 0.05$), and not different from that in the group fed without nougat (72.7%; $p = 0.37$). In conclusion, we produced, on a pilot scale, innovative sugar-free nougat with improved texture and similar sensory properties, in comparison with the traditional product. *In vivo*, the experimental nougat did not increase the diabetes incidence significantly.

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Introduction

Nougat is a sweet confectionary product made of whipped egg white, sugar, honey, and nuts. In a typical recipe, the honey-sweetened candy is flavoured with vanilla and almond extracts, and packed with toasted almonds. Traditionally, nougat is banned from the diets of diabetics. Diabetes mellitus is considered one of the most common chronic diseases worldwide, and recognised as one of the leading causes of morbidity and mortality (Chen *et al.*, 2022). The prevalence of diabetes has been increasing worldwide since 2000, with 463 million people aged 20 - 79 years having diabetes worldwide in 2019, corresponding to a prevalence of 9.3% (IDF, 2019). Furthermore, obesity and overweight represent a serious health concern worldwide. Consumer demand for healthy food is ever increasing, thus justifying the constant growth of the sugar-free confectionery market for diabetics and dieters.

Nougat remains a traditional food in several countries. In particular, it was included in the list of "Traditional Italian Food Products" (Italian Ministry of Agriculture, Food and Forestry act. 2081/92). In traditional manufacturing process, honey and sugars are warmed in a specialised boiler (*torroniera*), and uniformly mixed. Then, fresh whipped egg white is added and mixed again. Finally, nuts, generally almonds, and flavour are added to the *torroniera* (Speziale *et al*., 2010a). Structurally, nougat can be found in either chewy or grained form, depending on the state of the sugar matrix that is produced; crystals are not formed in the chewy version, whereas crystallisation occurs in the grained form. Therefore, chewy nougat shows an amorphous sugar phase with protein-stabilised air bubbles and fat globules dispersed throughout. Grained nougat also contains

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numerous small sugar crystals dispersed within the amorphous sugar matrix (Hartel and Nowakowski, 2017). The amorphous phase, along with the water content, plays a primary role in determining the texture of nougat (Ergun *et al*., 2009).

The development of a sugar-free alternative to this traditional foodstuff is therefore particularly challenging. In recent years, significant research in food science has been focused on the production of different colloidal systems with the aim of improving rheology and increasing the functional and nutritional properties of foodstuff. In this context, polysaccharides have enormous potential owing to their strong hydrophilic nature (hydrocolloids) (Douglas Goff and Guo, 2019). They form a colloidal dispersion which can modulate different rheological properties of the food system, thus influencing both textural and sensorial parameters (Yang *et al*., 2020), as well as tailoring the nutritional profile. Notably, their use can provide health benefits by regulating different parameters, such as the glycaemic response, immunity, and the gut microbiota composition, thus contributing to the prevention of various metabolic and food-related diseases (Yang *et al*., 2020).

All hydrocolloids behave as thickeners. In particular, hydrocolloids that provide high viscosity at low concentrations are preferred in food formulas. Among them, inulin and xanthan gum have been widely used in food applications (Habibi and Khosravi-Darani, 2017; Raungrusmee *et al.*, 2020). The technological use of inulin is mainly related to its properties as a fat replacer and texture modifier. It can form microcrystals, which interact with each other, thus forming small aggregates that agglomerate into a gel network (Hébette *et al*., 1998). Inulin has been evaluated as a partial sucrose replacer in short-dough cookies, thus giving no detrimental effect on consumer perception (Laguna *et al*., 2013). Xanthan gum is a hetero-polysaccharide of microbial origin authorised by the U.S. Food and Drug Administration (FDA) for application as a food additive without any restrictions. Xanthan gum disperses in cold and hot fluids by producing highly viscous solutions even at low concentrations, compatible with salts, and stable in acidic and alkaline solutions (Habibi and Khosravi-Darani, 2017).

In the present work, we aimed at developing, on a pilot scale, innovative sugar-free nougat by using xanthan gum and inulin, in combination with erythritol, a high-intensity sweetener (Laguna *et al*., 2013). The innovative nougat was analysed for its textural and sensorial properties. Any residual diabetogenic effect was analysed in non-obese diabetic (NOD) mice, a classical *in vivo* model of diabetes (Aubin *et al*., 2022).

Materials and methods

Raw materials

Roasted almonds (170 calories/100 g; 13% protein, 13% carbohydrates, and 74% fat), erythritol, honey, inulin, xanthan gum, granulated white sugar, glucose syrup, palm oil, dried egg white proteins, and flavours were purchased from Chimpex Industriale S.p.A (Caivano-NA, Italy).

Production of control and sugar-free nougat

The control nougat formula consisted of granulated white sugar, glucose syrup, water, palm oil shortening, dried high-whip egg white, and salt. It was manufactured follwoing a proprietary protocol (Oliviero srl, Monteforte Irpino-AV, Italy). The experimental nougat was prepared as follows. Erythritol was dissolved in boiling water to a final concentration of 3% (w/v) in an industrial planetary mixer (Sottoriva SpA, Marano Vicentino-VI, Italy). Next, xanthan gum (3%, w/v) was added and completely dispersed; the mixture was then transferred to a classical *torroniera* (Figure 1A). A whipping phase was prepared separately by high-speed stirring of egg white and inulin (50%, w/v) in water, and subsequently added to the mixture. Residual erythritol was then poured into the *torroniera* (final concentration: 10%, w/v), and continuous stirring was conducted for 2 h more at 75°C. Finally, almonds (10%, w/v), vanilla, and almond flavours (0.1%, w/v) were distributed uniformly in the boiler mixture. The paste was recovered and moulded using wood frames, covered on both surfaces with rice paper, cut, and promptly packed.

Shear force analysis

The assessment of cutting force was conducted on sugar-free experimental nougat and two commercial samples, grained and chewy nougat (Oliviero srl) as controls. The force was measured by using an in-house-made instrument (Institute of BioEconomy-CNR, S. Michele all'Agige-TN, Italy) consisting of a frame assembled with a hydraulic piston with a capacity of 50,000 N. Values were expressed in kg/cm^2.

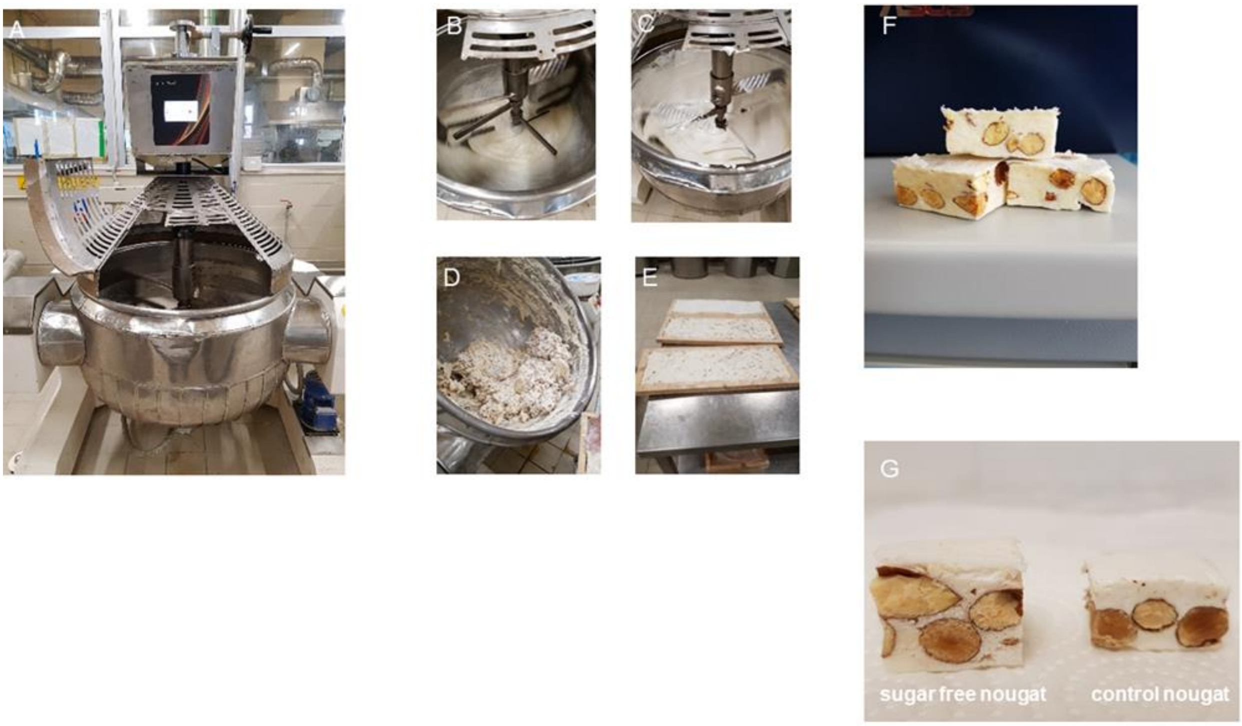

Figure 1. Pilot scale production of experimental nougat: **(A)** traditional *torroniera*; **(B)** mixing of xanthan gum and erythritol; **(C)** whipping; **(D)** addition of almonds; **(E)** paste moulded in wood frames; **(F)** appearance of experimental nougat; and **(G)** visual comparison between experimental and traditional chewy nougats.

Nutritional profile

Control nougat data were obtained from the nutrition facts label reported on a package of chewy nougat (Oliviero srl). Sugar-free nougat data were obtained from the nutritional tables from the US Department of Agriculture (USDA), with the exception of inulin, which is a fibre, and moisture. In particular, inulin caloric intake was estimated by considering that it is a non-digested but fermented oligosaccharide (Bhanja *et al.*, 2022). Moisture was calculated by adopting the Loss on Drying (LoD) method using an MB90 moisture analyser (OHAUS Europe GmbH, Nänikon Switzerland). LoD is the weight of water and any volatile matter that can be driven off under specified conditions, expressed as a percentage of the total (w/w).

Sensory evaluation

Descriptive sensory analysis (DSA) with a trained panel was used to discriminate and quantify the main sensory attributes of nougat. A panel of 10 people, aged 30 - 63 years (five female and five male, all technicians or researchers of the Institute of Food Sciences, CNR), with certified past experience in sensory evaluation of food, was enrolled. Based on literature data, 12 features were identified (Speziale *et al*., 2010a). Panellists were trained in two orientation sessions by assessing three commercial chewy nougat samples manufactured by Italian companies, as standards. This session was used to identify the attributes of the standards and assign scores for each one on a scale of 0 - 10 (0 = no appreciation; 5 = regular; and 10 = extremely intense) (Meilgaard *et al.*, 1999). Measurements were performed in individual booths. After the orientation session, control and experimental nougat samples (n = 6) were presented and scored based on training experience. Scores were statistically evaluated as mean ± standard deviation (SD). The evaluation was repeated three times.

Animals and experimental design

NOD/ShiLtJ mice, (Charles River Laboratories, Milan, Italy) were kept under specific pathogen-free conditions, fed with standard diet (Mucedola srl, Settimo Milanese-MI, Italy), and had free access to drinking water. The diet consisted of cereal flour, skimmed milk powder, potato starch, toasted soy flour, soybean oil, dicalcium phosphate, calcium carbonate, sodium bicarbonate, magnesium oxide, sodium chloride, vitamin A (16000 IU), vitamin D3 (1400 IU), Fe (200 mg), Mn (60 mg), Zn

(75 mg), Cu (13 mg), I (1 mg), and sepiolite (250 mg). This diet has been tested previously, and found to have low diabetogenic activity.

Twelve-week-old female mice were randomly divided into three diet groups of 11 - 12 animals fed with standard diet +/- nougat (5 g/day), as follows: no nougat (n = 11), control nougat (n = 12), and sugar-free nougat (n = 12). All procedures met the guidelines of the Italian Ministry of Health, and were performed in compliance with relevant laws and institutional guidelines (D.lgs. 26/2014 and EU Directive 2010/63/EU). The Institutional Ethics Committee of Ministry of Health (DGSAF) approved the study (approval number n.553/2020-PR).

Initial non-fasting glucose levels were measured using a Glucometer One-Touch (Ortho Clinic Diagnostic, Milan, Italy). The mice were inspected for diabetes every week during the experiment. A non-fasting glucose concentration greater than 250 mg/dL (13.8 mM), or preferably a chronic increase greater than 300 mg/dL (16.7 mM) was considered when assigning the mouse to the diabetic group (Leiter, 2009). Diabetes onset was monitored for 43 weeks, at which time the remaining mice were sacrificed, and glycaemia evaluated. The cumulative diabetes incidence was expressed as mean ± SD.

Statistical analysis

Shear force results were analysed by ANOVA and Tukey's test. Sensory data were analysed by t-test. Significant differences were reported at $p < 0.05$. Survival curves in NOD mice were evaluated by Log-rank (Mantel-Cox) test using GraphPad Prism software (version 6; GraphPad Software, La Jolla, CA, USA). The chi-square test was adopted to compute the level of significance for the cumulative diabetes incidence ($p < 0.05$).

Results and discussion

Set up of an innovative gluten-free nougat

The ingredients normally used to make nougat are honey, glucose syrup, water, egg white, almonds, and flavours in adequate proportions. The traditional Italian nougat recipe consists of uniformly mixing and heating honey with glucose syrup into whipped egg white, and lastly adding almonds and vanilla flavour in a large boiler pan (*torroniera*; Figure 1A). The product is subsequently moulded to confer the desired shape using wood frames, and cooled to room temperature. Therefore, the average content of simple sugars is very high, thus hampering consumption of nougat in large amounts for different categories of people. However, nougat remains an important product for traditional confectionary.

In order to manufacture functional sugar-free nougat resembling classical nougat, we developed a protocol by mixing appropriate amounts of xanthan gum, erythritol, and inulin. In an exemplary manufacturing method, xanthan gum and erythritol were dispersed firstly with high shear in a planetary mixer in order to produce thickened foam that was poured into the *torroniera* (Figure 1B). Then, an innovative whipping phase was adopted by mixing egg white, inulin, and residual erythritol. This mixture was added to the foam in the *torroniera* (Figure 1C). The suspension was stirred at uniform speed for 2 h at 75°C to produce a nougat-like paste. Finally, toasted almonds were added and mixed for 10 min (Figure 1D). The obtained paste was recovered and moulded in typical wood frames (Figure 1E). The formed nougat was creamy but not sticky, and it could be cut easily by mechanical means (Figure 1F). We found that the appearance of experimental nougat was similar to that of control nougat (Figure 1G), and also as previously described in literature (Speziale *et al*., 2010a).

In Figure 2, we show the shear stress resistances (kg/cm^2) of a traditional chewy almond, a traditional grained, and the experimental sugar-free nougat. As expected, the latter was found to be similar to the traditional chewy nougat.

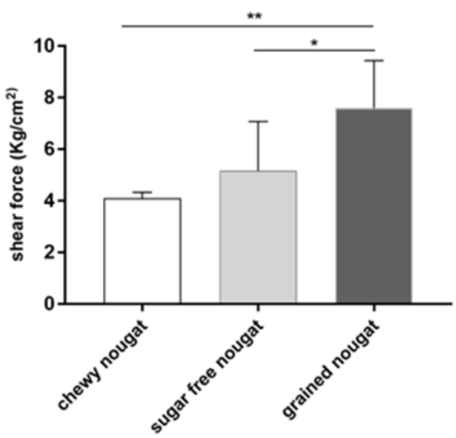

Figure 2. Shear forces (kg/cm^2) of two control (chewy and grained) commercial nougats and of experimental sugar-free nougat. Columns represent mean ± SD of six measurements (n = 6). Results are representative of three different experiments. *p < 0.05; **p < 0.01.

Nutritional parameters

The nutritional profiles of control chewy nougat and experimental sugar-free nougat are reported in Table 1. A dramatic decrease in the carbohydrate content and the caloric intake characterised the experimental nougat. Furthermore, the carbohydrate component was mainly attributable to non-digestible erythritol, whereas the highly increased fibre content in the experimental nougat was due to the inulin component. Importantly, the amount of inulin in 100 g of product was in line with a daily dose, which was reported to induce different positive biological effects (Wan *et al*., 2020).

Table 1. Nutritional profile of control and sugar free nougats.

100 g	Control nougat*	Experimental nougat**
Moisture	2 g	4.1 g
Energy	477 kCal	190 kCal
Protein	6.4 g	7.2 g
Fat	20 g	12.3 g
Carbohydrate	66 g	50.8 g
Fibre	< 1 g	26 g

*Oliviero srl; **USDA.

Descriptive sensory analysis

Descriptive sensory analysis (DSA) has been used successfully for comparing different attributes of foods (Yang and Lee, 2019). Herein, the panellists focused on the following classical sensory attributes: nougat colour; almond content and size; flavour (almond, vanilla, and honey); sweetness; and texture (hardness, fracturability, cohesiveness, chewiness, solubility, and adhesiveness) (Speziale *et al*., 2010a; 2010b).

As shown in Figure 3, the sensory profile of commercial nougat was characterised by a high intensity of sweetness, moderate intensity of vanilla flavour, and light intensities of almond and honey flavours. Furthermore, the texture profile of control nougat was characterised by moderate values for all the different parameters, with the exception of higher cohesive texture. These data were well in line with previous findings (Speziale *et al*., 2010a). Flavour features were preserved in sugar-free experimental nougat, with the exception of less intense vanilla flavour. Notably, sweetness was unchanged, whereas texture parameters changed significantly. In particular, experimental nougat showed reduced cohesiveness, which is the force between food particles, and that which increases fracturability. Both changes were expected, considering that we introduced deep modifications in the confectionary mixture. On the other hand, these changes positively influenced two other texture parameters: the solubility was significantly increased, making experimental nougat easier to dissolve in saliva; the adhesiveness, which is the work needed to detach nougat from the teeth or palate, was significantly reduced. The latter feature certainly represented an important improvement by considering the need to provide healthier confectionary products.

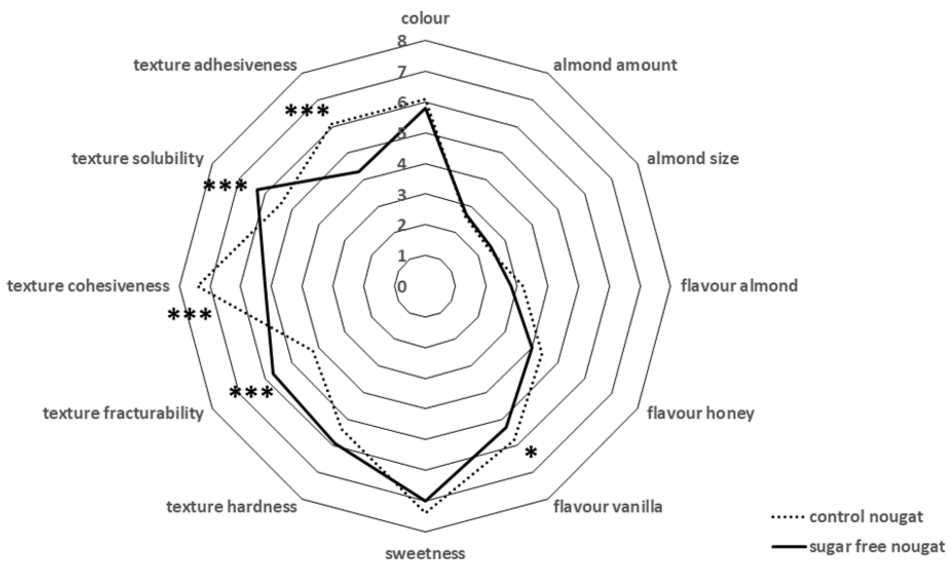

Figure 3. Descriptive sensory analysis of appearance, flavour, and texture features of control (chewy) nougat (dotted line) and experimental sugar-free nougat (continuous line). Values are means of six evaluations from ten panellists, and representative of three independent tests. *$p < 0.05$; ***$p < 0.001$.

Assessment of diabetogenic effects in vivo

The importance of dietary treatment in diabetes is well known. Given that the glycaemic response to foods cannot be predicted simply based on their chemical composition, before advice can be given to diabetics, data on the *in vivo* physiologic effects of experimental nougat are mandatory. To this end, 12-week-old female NOD mice were divided into three intervention groups (*n* = 6 - 9): one group was subjected to a previously established low diabetogenic diet (no-nougat group); another group was fed with a diet supplemented with control chewy nougat (nougat group; 5 g/day); the third group received a diet supplemented with experimental nougat (sugar-free nougat group; 5 g/day).

During the treatment, diabetes onset was monitored by analysing non-fasting glycaemia, starting from the 12th week (Figure 4A). Figure 4B shows the glycaemic trend in the three diet groups. The results indicated that the control chewy nougat-based diet significantly increased the onset of diabetes in comparison with the other two diets. Notably, the sugar-free nougat group did not differ significantly from the no-nougat group. The end-point analysis of glycaemia is shown in Figure 4C. The number of mice that did not develop diabetes in the no-nougat group was 72.7% as compared to 66.6% in the sugar-free nougat group and 33.0% in the nougat group. The difference between the nougat diet and the other diet groups was significant ($p < 0.05$), while the control and sugar-free group were not different ($p = 0.37$). These results unequivocally demonstrated that sugar-free nougat was unable to increase the incidence of diabetes in NOD mice.

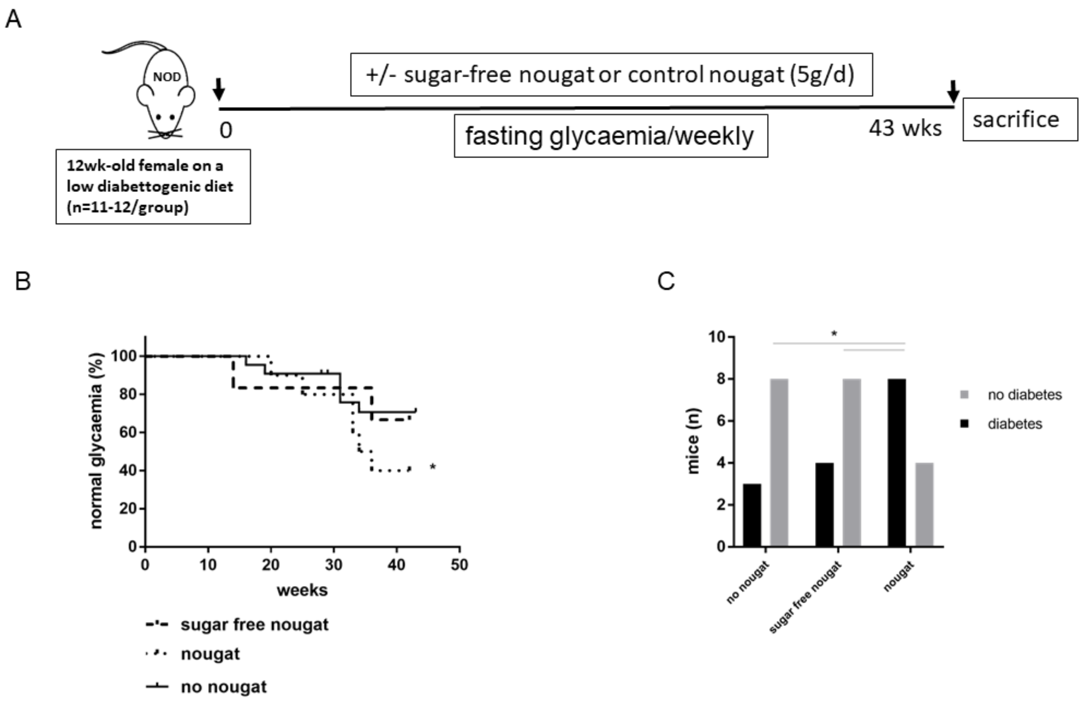

Figure 4. *In vivo* study in NOD mice: **(A)** time schedule of diet administration and analysis; **(B)** onset of diabetes; 12-week-old female NOD mice received one of three different diets for 43 weeks (*n* = 11 - 12 mice/group); diabetes was determined based on non-fasting blood glucose levels; and **(C)** diabetes incidence; columns represent the number of mice showing hyperglycaemic (black columns) or normal glycaemic values (grey columns) at the end of each diet regimen. *$p < 0.05$.

Conclusion

The average content of simple sugars is very high in traditional nougat, thus limiting its widespread consumption. With the aim of developing functional sugar-free nougat, we set up an industrial protocol by mixing xanthan gum with erythritol and inulin. Both technological and sensory parameters, as well as the *in vivo* health assessment, confirmed the usefulness of the new confectionary foodstuff. Taken together, our results could pave the way to the use of functional confectionery products, improved from a health point of view, thanks to the application of highly innovative food components.

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