

Obtention and characterization of dried gels prepared with whey proteins, honey and hydrocolloids mixture

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Abstract

BACKGROUND: Large amounts of honey and liquid whey derived from the dairy industry are produced in Argentina. Honey is exported in bulk and whey is transformed into whey protein concentrates and isolates. The objective of this work was to investigate the effect of pH, composition and storage time on the properties of dried gels with honey, whey proteins and hydrocolloids.

RESULTS: Colour properties varied according to pH and composition. The fracture stress of the dried gels prepared with corn starch was higher than those prepared with guar gum in all conditions assayed. The Young's modulus was higher at pH 7 for both compositions and increased with storage time. Rubbery characteristics were found in dried gels with guar gum, while corn starch and guar gum made the microstructure rougher. Multivariate analysis showed that samples could be grouped by pH. Panellists preferred pH 7 products over acidic ones and no significant differences in the sensory properties were found using either corn starch or guar gum in the formulation.

CONCLUSIONS: Results demonstrated that it is possible to generate a new product, which may open new applications for honey and whey in food formulations.

Keywords: Dried gels, Honey, Whey Proteins, Corn Starch, Guar gum

INTRODUCTION

Snacks, whether sweet or salty, are part of the diet of most people. Currently the term "snack" refers to a product easy to eat, accessible, small, solid or liquid, requiring little or no preparation and is intended to satisfy hunger between meals. Snacks include a broad range of products in many forms, such as sandwiches, yogurt and even ice creams.¹ Although a large number of products could be considered as snacks, french fries, potato chips, pretzels and other extruded products are the most popular. These kind of products often contain significant amounts of sweeteners, preservatives, flavourings and salt, and they are classified as "junk food". The change in lifestyle of consumers and

their health awareness have encouraged scientists and the food industry to change formulations and ingredients to increase the nutritional value and safety of foods.² Originally, snacks were flavoured with chocolate and peanut butter, but the current trend is towards more variety and sophistication, both to attract consumers and to differentiate products in the marketplace.³ With the introduction of new fat substitutes and natural ingredients such as phytochemicals, mixtures of grains, fruits, honey, vitamins, proteins and antioxidants, snack image has changed. Snack products is a market area that is growing worldwide. Demand is driven primarily by taste and health considerations and consumers are not willing to compromise on either (<http://www.nielsen.com/us/en/press-room/2014/global-snack-food-sales-reach-374-billion-annually.html>).

Whey proteins provide nutritional benefits such as lowering the energy content of foods when used as fat substitutes, raising the protein level, and balancing the amino acid profile. The ability of whey proteins to form gels under heating conditions is one of their main functional properties.⁴ Gels improve the performance of various food products by enhancing their appearance and texture. Moreover, gel properties are strongly dependent on pH, presenting a more aggregated structure at pH 4 (near the isoelectric point of whey proteins). Noncovalent interactions are involved in the structure of acidic gels, whereas at neutral pH, intermolecular sulfhydryl-disulfide interchange reactions are favoured. Therefore, the texture of acidic gels is different from the texture of pH 7 gels.⁴ The presence of other components also affects gel properties as interactions between whey proteins with, for example, hydrocolloids could modify the cohesive energy that holds the molecules together in the gel structure. Yamul et al.⁵ found that wheat flour increased the strength of cross-links in the whey proteins gel structure.

Honey, as a natural mixture of sugars, is attractive to food processors, not only because of its flavour and bodying characteristics, but also due to its general stability and storability, when properly processed.⁴ Argentina has had a significant development in the art of beekeeping and produces large amounts of honey which is exported in bulk. In previous works^{6,4,7} honey was added to whey protein concentrate gels in order to obtain a gelled product with different characteristics of taste, aroma and texture.

Based on what is mentioned above, dehydration of whey protein gels with honey could result in a snack-type product that is appealing to consumers. Hot air dehydration has been historically used to remove moisture from food stuff, extending its shelf life and avoiding microbiological spoil.⁸ However, during drying the product could be damaged and the addition of hydrocolloids could help maintain its structure. Over the years, this process has been spreading its field of application, not only to extend the shelf life of a food, but also to generate particular characteristics of texture, aroma and flavour in the dehydrated product.⁹

Therefore, the objective was to analyse the structural, functional and sensory properties of dried gels prepared at different pHs and compositions.

MATERIALS AND METHODS

Materials

Whey protein concentrate (WPC) was kindly provided by Arla Foods Ingredients SA. (Argentina). The WPC contained 777.1g kg⁻¹ protein (N x 6.38), 57.4g kg⁻¹ moisture, 27.7g kg⁻¹ ash, 38.3 g kg⁻¹ lipids and 99.5 g kg⁻¹lactose (estimated by difference). Honey was harvested in 2013 from apiaries located in the city of La Plata , Buenos Aires province (Argentina) and the composition was: 393.0 g kg⁻¹Fructose, 337.0 g kg⁻¹glucose, 21.0 g kg⁻¹sucrose, 7.0 g kg⁻¹ash and 157.0 g kg⁻¹ moisture. Corn starch (Maizena, Unilever, Argentina) and Guar gum (Lodra SRL, Argentina) were also used.

Snacks preparation

Aqueous dispersions (150 g kg⁻¹ protein, 300g kg⁻¹ honey, 50 g kg⁻¹ corn starch or 10 g kg⁻¹ guar gum) of WPC-honey-hydrocolloids (corn starch or guar gum) were adjusted to pH 3, 4 and 7 with HClor NaOH. Dispersions were placed in glass tubes (1.6cm i.d.×6 cm height) with tightly closed stoppers. Gelation was carried out by heating the tubes in a waterbath (Dalvo, Ojalvo SA, Argentina) at 90°C for 30 minutes. After heating, the tubes were cooled to room temperature and kept at 4°C for at least 15 h. before further processing. Gels were cut into 0.5 cm thick discs and dried at 85°C for 100

minutes in an automatic controlled mechanical convection oven (San Jor, Argentina) to obtain the dried gels.

Water Activity and moisture content

The water activity was measured with a Water Activity Meter Aqualab series 4 (Decagon Devices Inc., Washington, USA). The moisture content was measured according to AACC 44–19 (AACC International, 2000). Both measurements were performed in triplicate and the values presented are the average of the determinations.

Colour

Superficial colour was measured with a Chromameter CR 300 Minolta (Osaka, Japan) and Hunter parameters (L^* , a^* and b^*) were determined. Values are the average of at least five determinations.

Texture

Samples of dried gels were stored in plastic bags at room temperature for 60 days and fracture properties were studied by a three-point bending test with a texture analyser (TA-XT2i, Stable Micro Systems Ltd, United Kingdom) at days: 0, 3, 7, 15, 30, 45 and 60. The span length (L) was 0.7 cm and the compression speed was set at 1 mm/sec. The force F (Newton) needed to break the snack and the distance travelled by the probe before breaking the sample (Y ,cm) were obtained from the graph F vs. time.¹⁰ The width d (cm) and thickness b (cm) of snacks were measured using a Vernier calliper. All these parameters were used to calculate the fracture stress σ (N/m^2), the fracture strain (ϵ) and the Young's modulus E (N/m^2). Values are the average of at least three determinations.

$$\sigma = 3FL/2db^2 \quad (1)$$

$$\epsilon = 6bY/L^2 \quad (2)$$

$$E = L^3s/4db^3 \quad (3)$$

Where s is the tangent of the first part of the curve F vs. time.

Microstructure

Optical microscopy

A small amount of gel (with or without 50 g kg⁻¹ corn starch or 10 g kg⁻¹ guar gum at pH 3, 4 or 7) was extended over a microscope slide, dried, cooled at room temperature, covered with a cover glass slip and observed at 10× with a microscope Leica DM 2500 (Leica Microsystems, Germany).

Confocal microscopy

Observations were carried out on a Leica TCS SP5 Confocal Laser Scanning Microscopy (Leica Microsystems, Germany). The He/Ne visible light laser was used at a power of 30%. The following Leica objective lens was used: 63×1.4 numerical aperture with a zoom of 1.7. A mixture of rhodamine B (0.01 g kg⁻¹) and fluorescein isothiocyanate (FITC) (0.1g kg⁻¹) in distilled water was used for non-covalent labelling. FITC will preferentially label starch and Rhodamin B will preferentially label protein, but (to a lesser degree) Rhodamin B can also label starch, and FITC can also label protein.¹¹ Dyes were added to aqueous dispersions (before gelation of the sample) to ensure a complete penetration into the whole sample structure. After gelation, a sample of gel was placed over a microscope slide, dried, cooled at room temperature and covered with a cover glass slip. Samples were kept in darkness until observation. The excitation wavelengths were 488 nm (FITC) and 568 nm (rhodamine B) and the emission wavelengths were 518 nm (FITC) and 625 nm (rhodamine B). Digital image files were acquired in 1.024×1.024 pixel resolution and analysed with LAS AF LITE software (Leica Microsystems, Germany). Reported images were recorded at a penetration depth of 15 μm.

Sensory evaluation

Panellists were recruited from those who work at the National University of La Plata who declared that they were not hungry at the moment of the test. The test samples were presented to 34 non-trained panellists in plastic containers coded with a three-digit number, according to a randomised

complete block design. The order of presentation was balanced and randomised to eliminate contrast effect and positional bias. The test was performed between 10 and 11 AM. The experimental environment was kept constant for all sessions and no outside influences were allowed to interfere with the subjects' assessments of the product. Panellists evaluated pH 3 and pH 7 snacks for colour, taste, texture and overall acceptability based on a nine-point hedonic scale (8 cm long) labelled at each anchor: (left anchor: 1 = extremely dislike; right anchor: 9 = extremely like). Results were expressed as a percentage of the total scale distance. Mineral water was used to clear the subjects' palates between samples.

Data analysis

An analysis of variance was performed and the least significant differences were calculated to compare the means at a level of 95% using the Fisher test. A p-value of less than 0.05 was considered significant.

Principal component analysis (PCA) and cluster analysis (CA) was performed to examine the relationships among physicochemical parameters (texture, colour, moisture content and water activity) and type of dried gel. Standardised data and varimax rotation was used for PCA. Only the principal components with eigen values higher than one were considered significant. The PCA model was validated by the cross validation method. Cluster analysis was performed on the standardised data. Clusters were calculated using the Euclidian distance and the Ward technique which allowed a better discrimination of the clusters.¹²

Data analysis was performed by using the Infostat software 2014e version (Córdoba National University, Córdoba, Argentina).

RESULTS AND DISCUSSION

Visual appearance and microstructure of snacks

The appearance of a food stuff influences consumer acceptance, even before the product is introduced into the mouth. Whether it is an herb-encrusted chicken breast, a low-fat snack cracker or crystal-clear vitamin-enhanced water, food technologists rely on ingredients that provide visual appeal to a product to encourage its consumption. Figure 1 show the product obtained after drying. Those products prepared at acidic pHs (Fig 1 a,b,d,e) show a coarser surface than the sample at a neutral pH. The use of guar gum or corn starch in the formulation did not distinctly modify the appearance of both products except at pH 7 in which the dried gels with corn starch were darker than those with guar gum. Dried gels with guar gum showed a much more uniform surface than those with corn starch at pH 3. Figure 2 shows the microstructure of dried gels observed by optical microscopy. Samples a, d and g were not very different from one another as a smooth microstructure is shown, except at pH 7 (Fig. 2g) in which dark areas corresponding to a porous structure were observed. On the other hand, the addition of corn starch or guar gum turned the microstructure rougher with non-uniformly spread pores throughout the sample. Probably during the drying at high temperature, water was removed rapidly from the structure of the gels leaving voids that did not collapse and this resulted in a porous structure. Starch granules are not observed in Figures 2 (b, e, h) because they were fully gelatinised at the conditions used in the preparation of gels (90°C for 30 min) and the granules probably completely lost their identity after drying the gel. Dried gels at pH 7 were browner than those prepared at acidic pHs, which could be due to the fact that Maillard reactions are favoured at a neutral pH.

Confocal microscopy images showing the microstructure of the dried gels can be seen in Fig. 3. Rhodamine B binds non-covalently to the hydrophobic zones of proteins, thus, the red coloured areas correspond to the fluorescence of the dye, indicating the presence of a whey protein network. In an aqueous dispersion of proteins and starch, a phase separation into protein-enriched and starch-enriched fractions might occur depending on the biopolymers concentration. Figure 3 (b,e,h) shows a two phase system with discontinued zones of fluorescence indicating the existence of whey protein aggregates (red fluorescence) and zones of gelatinised starch (green fluorescence). From these pictures, it is not possible to distinguish starch granules, as they were completely swollen after gelation and disrupted after drying. Similar observations were reported in a previous work.¹³ Gelatinised starch seems to refill

the hollows in the whey protein network making the structure more compact. Figure 3 (a,d,g) shows a uniform distribution of fluorescence in samples containing only whey proteins (without corn starch or guar gum) at all assayed pHs. On the contrary, samples containing corn starch (b,e,h) or guar gum (c,f,i) exhibited a rougher and coarser structure for all assayed conditions.

Colour, water activity and moisture content

Colour control is an invaluable tool to standardise food snack manufacturing and keep uniform colour during food processing, storage and distribution. Table 1 shows colour Hunter parameters of dried gels at different pHs and composition. Samples prepared at pH 7 were darker because the lightness (parameter L*) decreased when pH was shifted from acidic to neutral. This is probably due to the non-enzymatic browning reactions such as caramelisation or Maillard browning induced by the heat-moisture treatment. These reactions, which are favoured at neutral pHs, have been reported to contribute to the colour change.¹⁴ Similar results were obtained by Yamul et al.^{6,5} in whey protein-honey gels. On the other hand, the parameter a* (reddish) was higher at pH 7, except in dried gels with corn starch in which no significant differences ($p < 0.05$) were observed. The value of parameter b* (yellowness) was higher at acidic pHs. Those samples prepared with guar gum were significantly ($p > 0.05$) darker than those with corn starch, except at pH 3 in which the opposite effect was observed. The effect of composition was not significant ($p < 0.05$) on the parameter a* and b* except at pH 4 in which the b* value was smaller for dried gels containing guar gum.

Water in food systems, not only plays a role as a texturiser, but also influences the shelf life of the foodstuff. Water activity is a parameter used by food technologists to formulate shelf-stable foods. If a product is kept below a certain water activity, then mold growth is inhibited resulting in a longer shelf life. No significant differences ($p < 0.05$) in the water activity and moisture content were observed between dried gels with corn starch or guar gum at all assayed conditions (Table 1). On the other hand, dried gels at acidic pHs showed significantly lower values of water activity and moisture content than pH 7 samples in which a mold development was observed after 16 days of storage at both assayed compositions. Acidic gels have a weaker structure than pH 7 gels due to the non-covalent interactions

that are responsible for the gel structure^{6,5}, thus, during drying water evaporates easier in acidic gels. In contrast, covalent interactions in pH 7 gels create a steady and closed structure that strongly holds water molecules resulting in a product with a higher water activity after drying.

Textural properties

Fracture is the separation of a material into two or more pieces under the action of stress and it is related with the hardness of a material. Before fracture, some force vs time curves (Fig. 4) corresponding to snacks containing corn starch or guar gum showed a serrated profile which could be attributed to small fractures in the material. These results suggest an inhomogeneous structure of the snacks as shown in the images (b,e,h and c,f,i) of Fig. 2 and Fig. 3. Baltsalvias et al.¹⁰ also found a similar effect in the fracture properties of short-dough biscuits. Results in Fig. 5 (a,b,c) showed that the fracture stresses of snacks with corn starch were higher than those with guar gum at all assayed conditions. Starch gelatinisation increased the hardness of the product matrix¹⁵ as was confirmed by the more compact structure observed through the confocal analysis (Fig 3 b,e,h). The fracture stress increased with storage time at pH 3 or 4 (Fig. 5 a,b) until a maximum hardness was reached on day 7 and then decreased to values similar to those on day 0. Baier et al.¹⁶ also found a similar hardening pattern in stored protein bars, during the first four days the bars hardened and then softened thereafter. On the other hand, snacks prepared at pH 7 (Fig. 5 b,) either with guar gum or corn starch increased the fracture stress with storage time. Usually, in food systems hardening is a multifactorial phenomenon which depends on the composition, physicochemical changes of the components, interactions between components and storage conditions. Hardening could be explained considering that during storage there is a moisture migration away from protein particles towards the hydroxyl groups of the sugars resulting in further aggregation between proteins.¹⁷ According to Labuza et al.¹⁸, hardening starts with the formation of separate aggregates between tiny particles in the product structure and then the aggregation expands to the entire structure. The snacks of the present work contain glucose and fructose (polyols) from honey which favour the Maillard reactions. Liu et al.¹⁹ suggested that hardening is influenced by the polyol used in the formulation of a whey proteins

enriched product and by the interactions between proteins and polyols. Therefore, the Maillard reaction would also be involved in hardening. However, Mc Mahon et al.²⁰ demonstrated that despite the Maillard browning that occurs during storage in a high protein content product, it is not the mechanism by which hardening occurs. Snacks prepared with corn starch were harder than those with guar gum, since, in addition to the factors mentioned above, starch retrogradation increased the fracture stress of the product (Fig. 5 a,b,c).

The Young's modulus (or modulus of elasticity) is the resistance of an object to deformation and it is a measure of the stiffness of the product.²¹ Results in Figure 5 (d,e,f) shows that pH 7 snacks were much stiffer than snacks prepared at acidic pHs for both compositions (corn starch or guar gum). Also, storage time increased the Young's modulus, especially at a neutral pH.

Products that can be reduced to small particles by the action of a small amount of pressure or friction are classified as friable. The friability can be correlated with the fracture strain; that is, the lower the fracture strain the more friable the product. The fracture strain of snacks (Fig. 5 g,h,i) prepared with guar gum at pH 3 and 7 was higher than those with corn starch, suggesting that guar gum makes the product less friable with rubbery characteristics. Mandala et al.²² found a rubbery crust in breads containing guar gum and attributed this effect to the water binding capacity of the hydrocolloid which influences the plasticity of the product. Similar values of the fracture strain were observed in snacks prepared at pH 4 for both compositions indicating similar textural properties for both types of snacks. The fracture strain of acidic snacks increased with storage time, except at pH 3 with corn starch in which no differences were found. During storage, environmental moisture uptake probably took place, which would explain the increase in rubbery texture. On the other hand, storage time at pH 7 did not significantly modify the fracture strain of snacks prepared with guar gum or corn starch. Results also showed that snacks prepared at pH 4 and 7 with guar gum were more friable in most cases than those at pH 3.

Multivariate analysis

Principal component analysis allows simplifying the study of several variables by combining them to reduce the number of parameters. Results in Figure 6a indicate that this analysis explains 86% of the variance among samples with the first two principal components (PC). PC1, which explains 66.4% of the variance, was mainly defined by L^* and b^* with positive correlation and the Young's modulus together with a^* , moisture content and water activity, which were opposite to the aforementioned (negative correlation). Thus, most of the variability on the physical properties of snacks can be explained by these variables. On the other hand, PC2 accounts for 22.5% of the variance and is mainly defined by hardness, fracture stress and fracture strain. The biplot (Fig. 6 a) shows the effect of variables on the principal components. Snacks prepared at pH 3 for both compositions, are mainly characterized by L^* and b^* . On the other hand, at pH 7 for both compositions are characterized by the Young's modulus, water activity, moisture content and a^* . Fig. 6 b shows that snacks were clustered into three groups which correspond to the different assayed pHs. The number of pre-defined groups usually relates to the number of significant PCs.²³ However, in our case, only two PCs were significant (eigenvalues higher than 1). The further away the variable was from the origin point, the larger the contribution of that variable to the PC model.²³ Fracture stress, Young's modulus, water activity, moisture content, a^* , b^* and L^* have the same contribution towards the PC separation since they show similar distances from the origin point. The results suggest that these variables contribute to the model with the same weight. On the other hand, fracture strain showed a shorter distance from the origin point.

Figure 6b shows the cluster analysis with an unrandom sample arrangement depending on the distance. At 75% (6.6) of the total distance, two clusters can be observed, one of them includes the snacks prepared at pH 7 and the other includes those prepared at acidic pHs. Closer to the origin point, at the middle point of the total distance (4.4), it is possible, with the variables analysed, to define three groups according to their pHs.

Sensory analysis

Consumer preference for snacks is largely determined by the sensory characteristics.²⁵ Figure 7 shows the sensory attributes (colour, taste, texture, and general acceptability) as a function of pH and composition. Significant differences ($p < 0.05$) were observed in these attributes between acidic and neutral snacks for all assayed conditions. On the other hand, no significant differences ($p > 0.05$) were observed in sensory attributes with guar gum or corn starch. According to the results of colour evaluation (Fig. 7 a), pH 7 snacks received the highest score. Food colour and visual appearance are of prime importance to capture consumer attention and are critical in the acceptance of the products.²⁶ Honey enhances the appearance of food systems specially when it is heated at pH 7, developing a brown colour which is perceived by consumers as an added value.

Honey, corn starch and guar gum are appreciated by food technologists due to their ability to modify the texture in food host systems. Texture can be defined as a sensation perceived by the sense of touch when food structure is deformed in the mouth.⁴ Fig. 7 b shows that pH 7 snacks were better ranked than acidic snacks, suggesting that consumers prefer friable characteristics (Fig. 4 i) in this kind of product.

The food industry aims to produce foods that are pleasurable to eat and differences in pH could lead to differences in taste perception for a given food. Results in Fig. 7 c indicate that pH 7 snacks obtained a higher score than acidic snacks.

Figure 7 d shows the general conclusion of the panellists suggesting that snacks prepared at pH 7 were better ranked than those at pH 3. It is important to note that panellists could find differences in snacks with guar gum or corn starch at any sensory descriptor evaluated. This information could be useful for the food industry to choose one hydrocolloid or the other for the formulation of the snacks.

CONCLUSIONS

Maillard reactions played an important role in determining the colour of pH 7 snacks. In addition, at pH 4 and pH 7 snacks containing guar gum were darker than those containing corn starch. The effect of composition (corn starch or guar gum) was not significant on the reddish (a^*) and yellowness (b^*) values except at pH 4.

Storage time affected the textural characteristics of the product mainly by hardening. Furthermore, snacks containing corn starch were harder than those containing guar gum. The pH also had an effect on texture, with pH 7 snacks being stiffer than acidic snacks. Moreover, guar gum made the product rubbery with low friability.

Multivariate analysis showed that samples could be grouped by pH and most of the variability on the physical properties can be explained by the colour parameters and Young's modulus.

The variations of structural properties were confirmed by microstructure observation using optical and confocal microscopy. Results indicated that the addition of corn starch or guar gum made the structure rugged.

A good sensory acceptance was observed, especially in pH 7 snacks, and no significant differences in the sensory properties were found using either corn starch or guar gum.

Results demonstrate that it is possible to generate a new product which may open new applications for honey and whey in food formulations. Further work with other hydrocolloids and/or beehive products should be performed to improve or expand the menu of snack options.

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REFERENCES

1. Sajilata MG and Singhal RS. Specialty starches for snack foods. *Carbohyd Polym* **59**: 131–151 (2005).
2. da Silva EP, Siqueira HH, do Lago RC, Rosell CM and de Barros Vilas Boas E.V. Developing fruit-based nutritious snack bars. *J Sci Food Agric* **94**: 52–56 (2014).

3. Aramouni FM and Abu-Ghoush MH. Physicochemical and sensory characteristics of no-bake wheat–soy snack bars. *J Sci Food Agric* **91**: 44–51 (2011).
4. Conforti P, Yamul D and Lupano C, Properties of biscuits and whey protein concentrate gels with honey, in *Functional properties of foods components* ed.by Lupano CE. Kerala, India, pp 93-108 (2007).
5. Yamul DK and Lupano CE. Whey protein concentrate gels with honey and wheat flour. *Food Res Int* **38**: 511-522 (2005).
6. Yamul DK and Lupano CE. Properties of gels from whey protein concentrate and honey at different pHs. *Food Res Int* **36**: 25-33 (2003).
7. Yamul DK and Lupano CE. Viscoelastic properties of whey protein concentrate gels with honey and wheat flour at different pH. *J Texture Stud* **40**: 319-333 (2009).
8. Vega-Mercado H, Góngora-Nieto MM and Barbosa-Cánovas GV. Advances in food dehydration. *J Food Eng* **49**: 271-289 (2001).
9. Klensporf D and Jelen HH. Effect of heat treatment on the flavor of oat flakes. *J Cereal Sci* **48**: 656-661 (2008).
10. Baltasvias A, Jurgens A and Vliet TV. Factors affecting fracture properties of short-dough biscuits. *J Texture Stud* **28**: 205-219 (1997).

11. Silva E, Birkenhake M, Scholten E, Sagis LMC and van der Linden E. Controlling rheology and structure of sweet potato starch noodles with high broccoli powder content by hydrocolloids. *Food Hydrocoll* **30**: 42-52 (2013).
12. Silvano MF, Varela MS, Palacio MA, Ruffinengo S and Yamul DK. Physicochemical parameters and sensory properties of honeys from Buenos Aires region. *Food Chem* **152**: 500-507 (2014).
13. Cassiani DM, Yamul DK, Conforti PA, Pérez VA and Lupano CE. Structure and functionality of whey protein concentrate based products with different water contents. *Food Bioprocess Tech* **6**:217-227 (2013).
14. Oli P, Ward R, Adhikari B and Torley P. Colour change in rice during hydration: Effect of hull and bran layers. *J Food Eng* **173**: 49-58 (2016).
15. Manley D, Classification of biscuits, in Manley's technology of biscuits, crackers and cookies, ed. by Manley D. Cambridge, London, pp. 271-278.
16. Baier SK, Guthrie BD, Elmore DL, Smith SA, Lendon CA and Muroski AR. Influence of extrusion on protein conformation and shelf-life extension of nutritional bars. Paper presented at the international symposium on the properties of water X, 2–7 September, Bangkok, Thailand (2007).
17. Loveday S, Hindmarsh J, Creamer L and Singh H. Physicochemical changes in a model protein bar during storage. *Food Res Int* **42**: 798–806 (2009).

18. Labuza T, Zhou P, Takerka H and Tran A. Evaluation of quality loss in model protein bars during storage. 6th Congress Euro Food Water Reims France. Available from: <http://www.eurofoodwater.eu/pdf/2010/Session3/>. Last accessed 30/12/2016.
19. Liu X, Zhou P, Tran A and Labuza TP. Effects of polyols on the stability of whey proteins in intermediate-moisture food model systems. *J Agric Food Chem* **57**: 2239–2245 (2009).
20. McMahon D, Adams S and McManus W. Hardening of High-Protein Nutrition Bars and Sugar/Polyol-Protein Phase Separation. *J Food Sci* **74**: 312-321 (2009).
21. Corradini MG and Peleg M, Solid food foams, in Food Material Science: Principles and Practice, ed. By Aguilera JM and Lillford PJ. Springer, New York, pp 137-145 (2008).
22. Mandala IG and Sotirakoglou K. Effect of frozen storage and microwave reheating on some physical attributes of fresh bread containing hydrocolloids. *Food Hydrocoll* **19**: 709–719 (2005).
23. Yücel Y and Sultanuğlu P. Determination of industrial pollution effects on citrus honey with chemometric approach. *Food Chem* **135**: 170-178 (2012).
24. Rohman A, Triyana K, Sismindari B and Erwanto Y. Differentiation of lard and other animal fats based on triacylglycerols composition and principal component analysis. *Int Food Res J* **19**: 475-479 (2012).

25. Kim EH, Corrigan VK, Hedderley DI, Motoi L, Wilson AJ and Morgenstern MP. Predicting the sensory texture of cereal snack bars using instrumental measurements. *J Texture Stud* **40**: 457–481 (2009).
26. Liu C, Liu W, Lu X, Chen W, Yang J and Zheng L. Potential of multispectral imaging for real-time determination of colour change and moisture distribution in carrot slices during hot air dehydration. *Food Chem* **195**: 110-116 (2016).

Figure 1

Visual appearance of snacks. pH: (a,d) 3; (b,e) 4; (c,f) 7. Composition: (a,b,c) 1%w/w guar gum, (d,e,f) 5%w/w corn starch. Protein content: 15 %w/w. Honey content: 30% w/w.

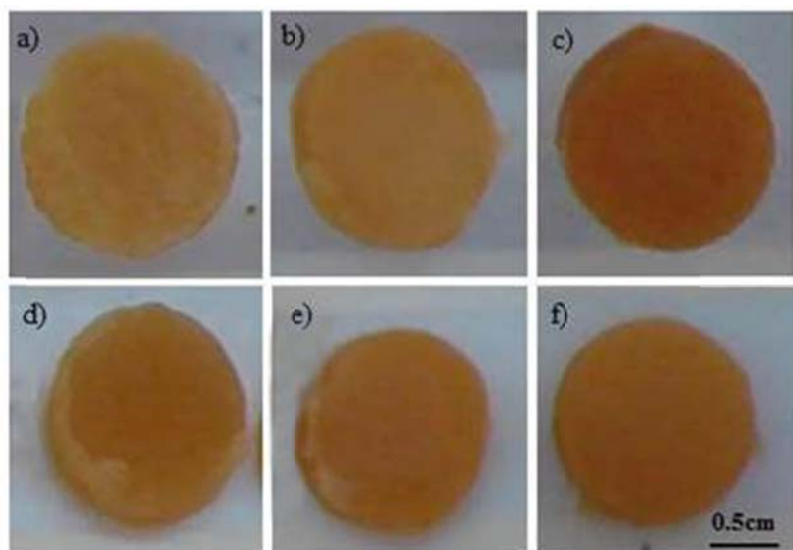


Figure 2

Microstructure of snacks observed at 10 \times . pH: (a,b,c) 3; (d,e,f) 4; (g,h,i) 7. Composition: (a,d,g) without hydrocolloids, (b,e,h) 5%w/w corn starch, (c,f,i) 1%w/w guar gum. Protein content: 15 %w/w. Honey content: 30% w/w.

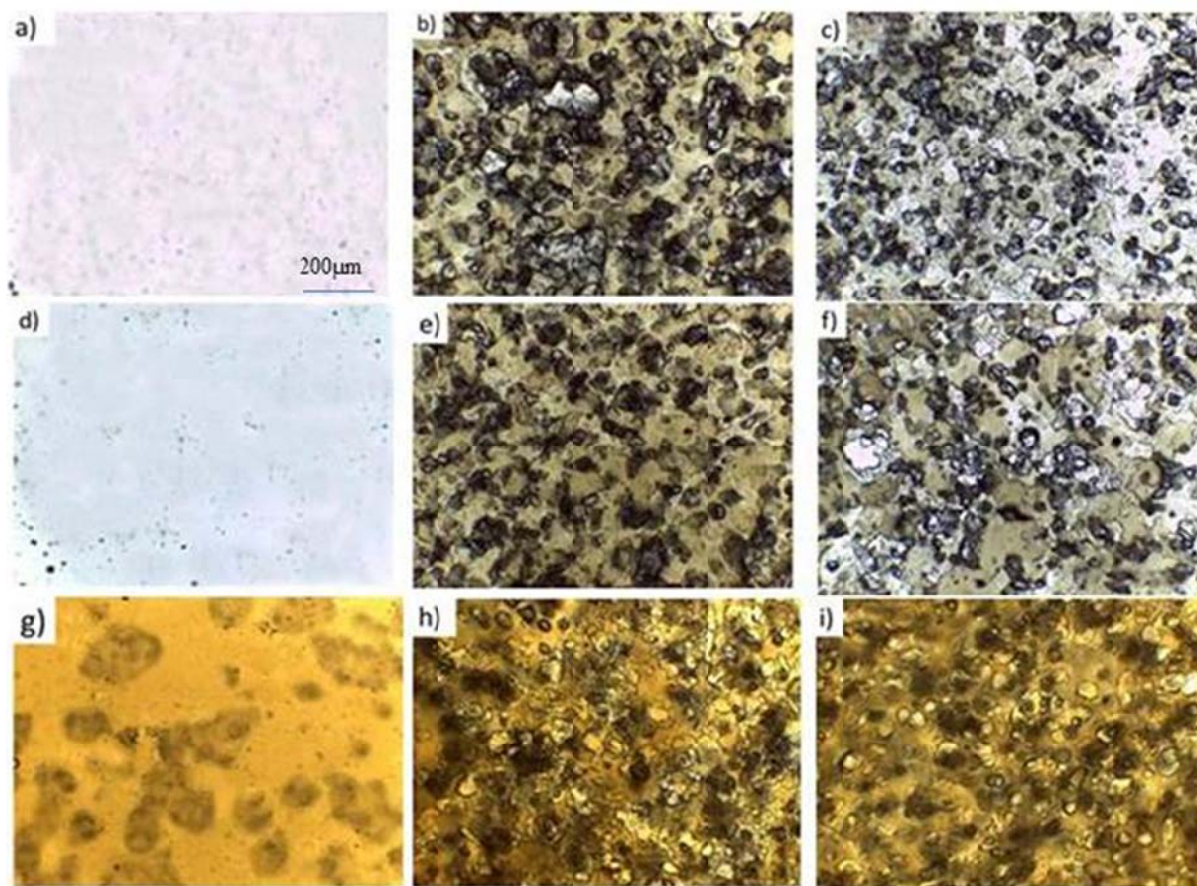


Figure 3

Microstructure of snacks observed by confocal laser scanning microscopy. Samples pH: (a,b,c) 3; (d,e,f) 4; (g,h,i) 7. Composition: (a,d,g) without hydrocolloids, (b,e,h) 5%w/w corn starch, (c,f,i) 1%w/w guar gum. Protein content: 15 %w/w. Honey content: 30% w/w.

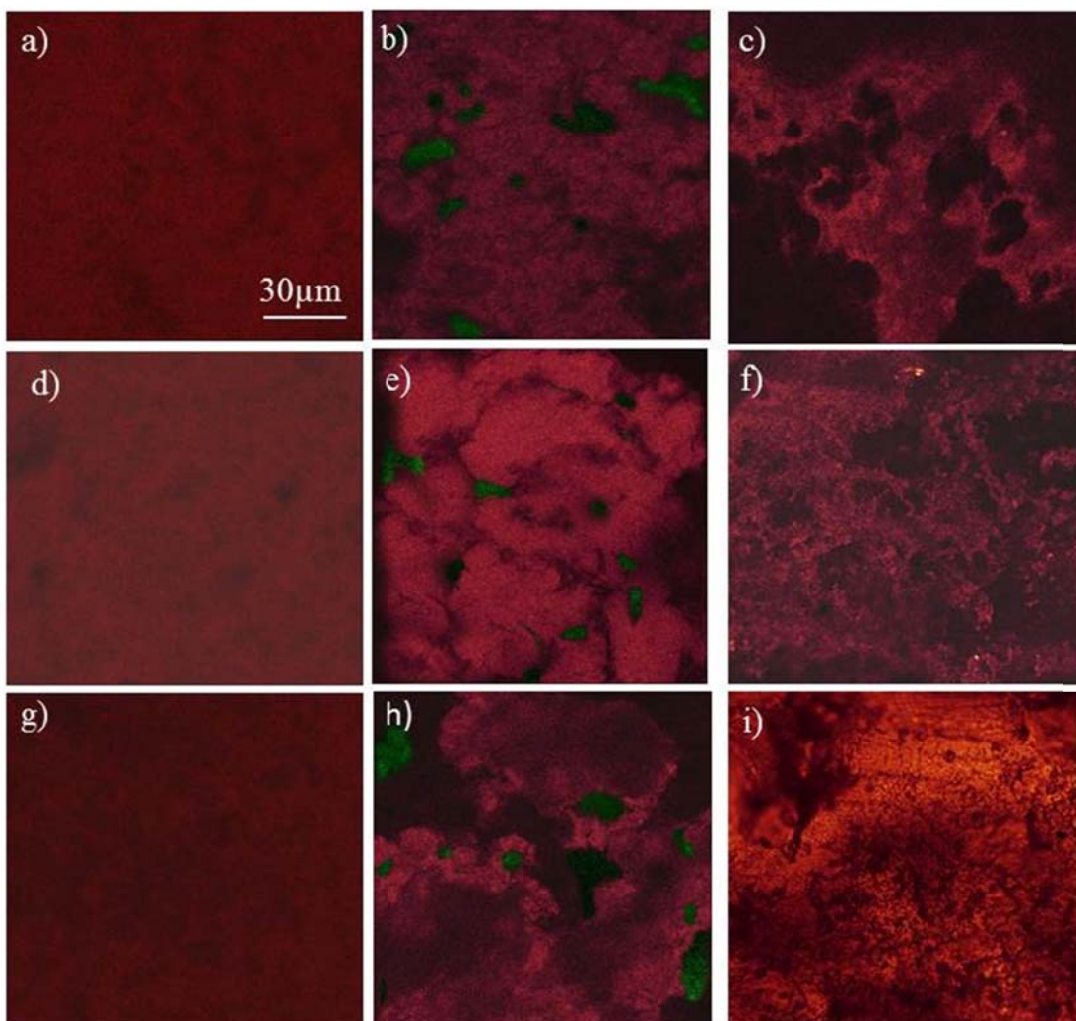


Figure 4

Example of a force vs distance representative experimental curve of a snack with guar gum (a) and corn starch (b) at pH 3 and 0 days of storage.

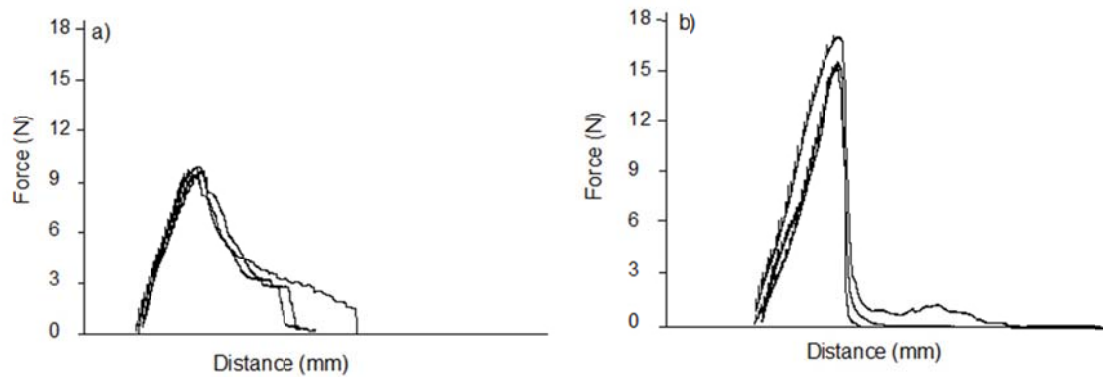


Figure 5

Texture properties of snacks prepared with corn starch and guar gum at different pHs and storage time. pH of snacks: (a,d,g) 3; (b,e,h) 4; (c,f,i) 7. Protein content: 15 %w/w. Honey content: 30% w/w. Corn starch snacks at all assay pHs and guar gum snacks at pH 7 showed mold growth after 50 and 16 days of storage, respectively.

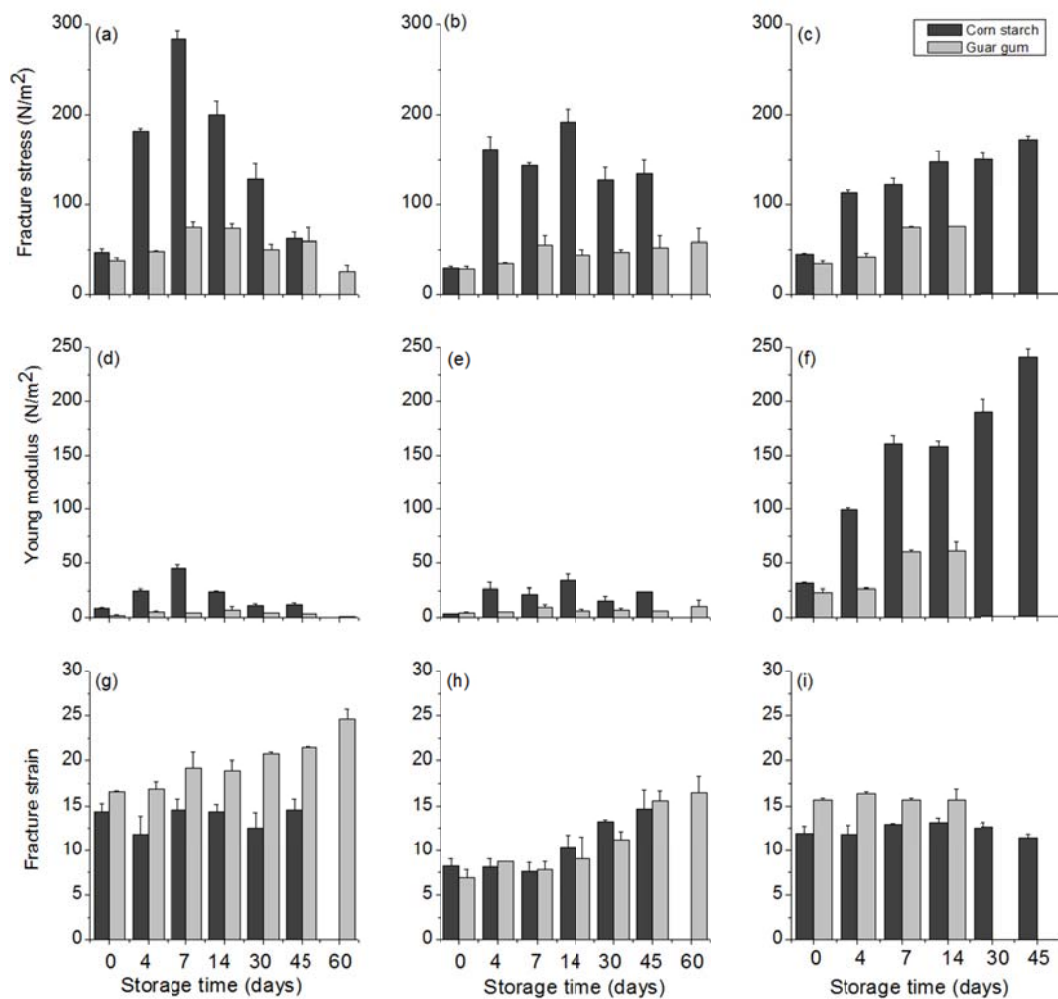


Figure 6

Multivariate analysis. a) Projection of physicochemical parameters of different snacks samples in the plane of the two principal components. b) Dendrogram of cluster analysis. CS: corn starch, GG: guar gum, a_w : water activity, (a^* , b^* and L^*) colour parameters.

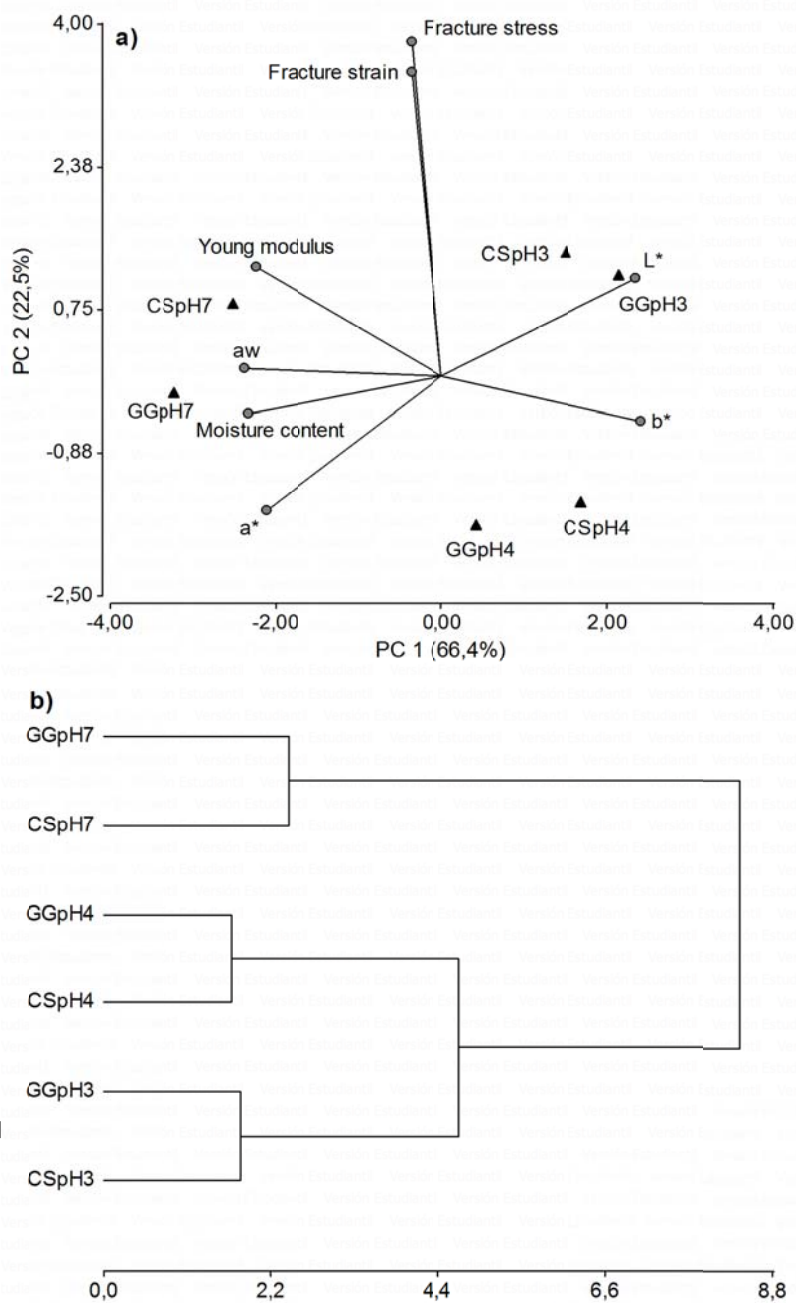


Figure 7

Sensory evaluation of snacks prepared with corn starch and guar gum at different pHs. Values with the same letter (capital letter for pH comparison, small letter for composition comparison) are not significantly different for each sensory attribute ($p > 0.05$).

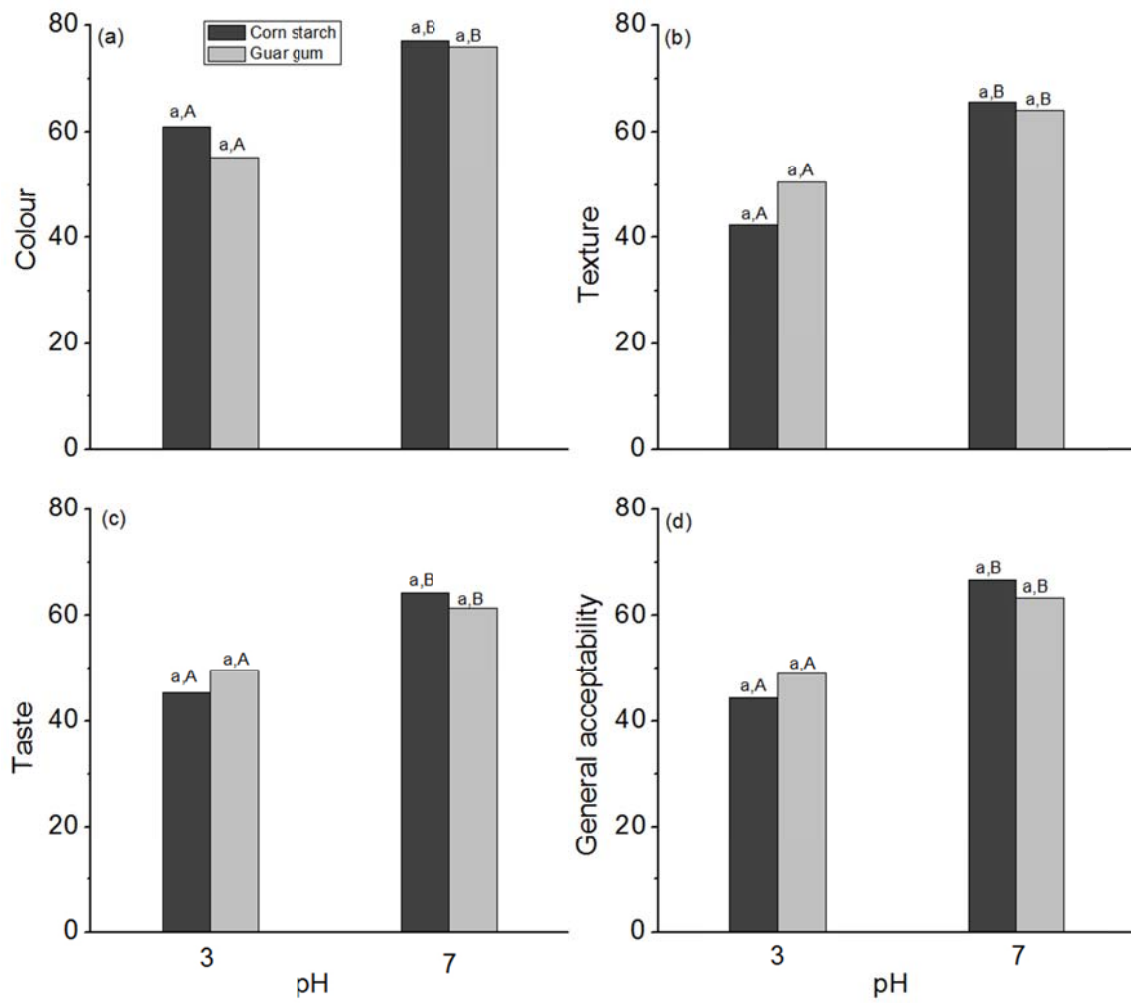


Table 1. Hunter colour parameters (L^* , a^* and b^*), water activity (a_w) and moisture content of snacks at different pHs and composition. Values with the same letter (capital letter for pH comparison, small letter for composition comparison) are not significantly different for each variable ($p>0.05$). CS: cornstarch, GG: guar gum.

pH	L^*		a^*		b^*		a_w		Moisture content (g kg^{-1})	
	CS	GG	CS	GG	CS	GG	CS	GG	CS	GG
3	62.28 ^{a,C}	65.91 ^{b,C}	6.72 ^{a,A}	5.65 ^{a,A}	26.39 ^{a,B}	26.31 ^{a,B}	0.536 ^{a,B}	0.558 ^{a,B}	266.2 ^{a,B}	275.7 ^{a,B}
4	60.16 ^{a,B}	57.09 ^{b,B}	7.62 ^{a,A}	7.35 ^{a,B}	28.38 ^{a,B}	23.89 ^{b,B}	0.533 ^{a,B}	0.639 ^{a;A,B}	267.7 ^{a,B}	304.5 ^{a,B}
7	52.68 ^{a,A}	49.26 ^{b,A}	8.03 ^{a,A}	9.37 ^{a,C}	17.35 ^{a,A}	16.82 ^{a,A}	0.792 ^{a,A}	0.746 ^{a,A}	326.9 ^{a,A}	371.4 ^{a,A}