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SCANNING ELECTRON MICROSCOPY STUDIES OF A TYPICAL SPANISH CONFECTIONERY PRODUCT: "XIXONA TURRON"

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Abstract

The microstructure of "Xixona turron", a typical Spanish confectionery product, made with toasted almonds, sugar and honey, was elucidated, in order to confirm the nature of those physical changes occurring in its manufacturing process, which has not been previously studied. The effect of the major components of "Xixona turron" (oil, proteins and sugar) on its structural change was established. The initial oil suspension of sugar syrup drops and proteins undergoes a phase inversion, becoming an oil/water emulsion when hot, and a solid-like structure, when the aqueous sugar continuous phase solidifies at room temperature. In order to determine the microstructure of "Xixona turron", toasted almond and "Xixona turron" samples were observed with the scanning electron microscope.

Key Words: Confectionery product; Microstructure; Scanning electron microscopy; Almond; Manufacture process; Heating treatment; Oil; Aggregated protein; Sugar matrix; Xixona turron; Inversion-phase phenomenon; Structural change.

Introduction

"Xixona turron" is a confectionery product, made with toasted almonds (64%) and concentrated syrup (80° Brix) of glucose, sucrose, honey and 1% ovalbumin. During the manufacturing process (Fig. 1), syrup is concentrated by heating until 80° Brix; then it is mixed with ovalbumin and almonds. When the mass is cold, it is crushed and the almonds broken, releasing oil. The product becomes a highly viscous suspension of syrup drops, proteins, and cellular fragments of almond, with a wide particle size distribution (Chiralt, et al., 1990). This suspension is then heated to 90 °C in a semi-spherical reboiler pan (0.65 m diameter), called a "boixet", where the product is also gently stirred. During this step, the product undergoes an important change in texture. The product entering in the "boixet" is a viscous suspension but because of the heating and stirring treatment its consistency increases suddenly at the end of this step, becoming a solid-like mass when cold. Then, it is cut and wrapped.

Previous studies (Chiralt, et al., 1990) lead to a hypothesis about the physical changes occurring in the "boixet" step: a similar phenomenon to the emulsion phase inversion. When the suspension is hot (at a temperature above the sugar glass transition, Tg), drops of liquid syrup (36% w/w of the product), almond proteins and cellular fragments are dispersed in the released oil (35% w/w of product) from the almonds; in fact the system is a complex water/oil emulsion. During the "boixet" step, at the end of the heating treatment, a structural change occurs due to coalescence and final aggregation of the syrup drops, which becomes the continuous phase of an oil/water emulsion. When the final product is cold, the continuous phase is solid, and oil, proteins and other cellular particles are trapped inside this new sugar matrix. At this moment the characteristic texture of the final product is achieved.

The aim of this work is to analyze the microstructure of the final product, in order to confirm the described hypothesis about the nature of physical changes occurring during the "boixet" step.
FORMULATION

- water
- honey
- sucrose
- dextrose

CONCENTRATION
(unti 80 °Brix, 107°C)

- ovalbumin
(1%, reconstituted)

MIXTURE

107°C
tasted almond
(64%)

CRUSHING
(stone roller mill)
room temperature

HEATING
(BOIXET, 30-90°C, 120 min.)

STANDING
(24 hours, room temperature)

PACKING

XIXONA
TURRON

Figure 1. Flow diagram of the "Xixona turron" process.

Material and Methods

Toasted Marcona variety almonds, the major variety used in the "Xixona turron" manufacturing process, and commercial "Xixona turron" samples (trade mark "Anti-Xixona", supreme quality), were observed with a scanning electron microscope (SEM). Cubes (5 X 5 mm) of each sample were cut with a razor blade and prepared in order to preserve protein structures.

Almond samples were fixed in 2% glutaraldehyde fixative solution with a phosphate buffer, pH 6.8 for 24 hours at 4 °C. After the fixation process, samples were dehydrated in a graded ethanol series of 10, 20, 40, 60, 80 and 100% (ethanol water v/v) for 20 minutes each, three 20 minutes changes in pure acetone, at room temperature, then samples were sliced to reveal a cut surface. Afterwards they were critical point dried with CO₂ in a Polaron E 3000 critical point drying apparatus. A Polaron E 6020 sputtering unit was used to coat the samples with gold, under a vacuum less than 10⁻² Pa, and 20 mA ionization current for 60 seconds.

"Xixona turron" samples for SEM observation were prepared in two different ways. In order to preserve protein structures, some of the samples were treated as described above for almonds. Sample cubes were directly fixed with vapors of osmium tetroxide in a hermetically closed desiccator, then sectioned and gold coated as described above. In this way, water soluble compounds, such as sugar, in the product were preserved.

Micrographs were obtained using an ISI DS-130 SEM operating at 20 KV. Stereo micrographs were made with a tilt angle difference of 20 degrees with a magnification range from 800 X to 6120 X.

Results and Discussion

As 64% of turron is toasted almonds, initial SEM observation of toasted almond microstructure will allow the identification of its cellular bodies in the final turron structure. Fig. 2 shows the cellular structure of toasted almond. The almond structure is neatly arranged as cells 20-40 μm in diameter. Protein bodies which make up 24% w/w, as albumins and globulins in 91% (Saura-Calixto, et al., 1982) are seen as relatively large spherical particles 1-9 μm in diameter. No starch granules were observed. Cytoplasmic matrix appears quite widely destroyed, probably due to a partial destruction of sample structure during roasting and the preliminary SEM preparation. Oil bodies or spherosomes (0.2-0.5 μm in diameter) are found in the cytoplasmic matrix (Aguilera and Stanley, 1990), but oil was not seen, having been removed by fixative solutions (ethanol and acetone) so that spherosomes are not present in the observed samples.

Glutaraldehyde fixed turron (Fig. 3A) shows a porous internal surface. The porous appearance can be partially due to loss of sugar and oil during the preliminary SEM preparation of the sample. Aqueous glutaraldehyde fixation will cause some solubilization of sugar and also oil will be extracted. So, only fixed proteins
Figure 2. Glutaraldehyde fixed toasted almonds. Protein bodies (pb) are packed in almond cells. Cell walls (cw), intercellular spaces (is) and residual cytoplasmic network (cn) are observable. Bar = 10 \( \mu m \).

Figure 3. (A). Glutaraldehyde fixed "Xixona turrón" showing a partially dissolved sugar matrix (sm) in which almond protein bodies (pb) are differentiated; bar = 10 \( \mu m \). (B) Higher magnification of the area indicated by an arrow in Fig. 3A, allows us to clearly observe an aggregate of protein bodies (ap); bar = 1 \( \mu m \).

Figure 4. (A). Osmium tetroxide fixed turron. Full oil holes (oh) and aggregated protein bodies (ap) are included in an amorphous sugar matrix (sm); bar = 10 \( \mu m \). (B) Higher magnification of the area indicated by arrow in Fig. 4A. Oil surrounding protein bodies in aggregates can be observed. Some aggregate protein bodies (ap) are preserved during cutting and appears totally (t) or partially (p) coated by continuous sugar matrix (sm); bar = 10 \( \mu m \).
and water insoluble compounds, like polysaccharide structures, are observable. The pores and holes in the structure could be the original places of oil or sugar. The semi-continuous structure was assigned to a residual sugar matrix, partially preserved by linked fixed ovalbumin or by a slow rate of solution during the glutaraldehyde fixation process. A large number of globular protein bodies are present, in many cases as aggregates. A higher magnification of this glutaraldehyde fixed surface (Fig. 3B) allows us to confirm the aggregated nature of these bodies.

The osmium tetroxide fixed sample of turrón (Fig. 4) shows a more preserved structure. A complex continuous sugar matrix can be observed, with holes of different sizes and shapes, which are full of oil or aggregated globular bodies, surrounded by an oil film; in some cases these holes are totally or partially coated by a film of sugar continuous phase. No crystallized sugar was observed, as expected from differential scanning calorimetry analysis (Galotto, 1989), which showed sugar glass transition. Aggregates of globular bodies, which also appear in glutaraldehyde fixed samples, are probably almond proteins, in the form of lipid-protein associations (Rand, 1976). They are probably present in the original oil suspension and remain in the product, when inversion of the system occurs in the "boixet" step. Flocculation of proteins in the oil suspension is logical, as oil is not a good solvent for proteins and an attractive-repulsive balance will causes protein segments to link (Dickinson and Stainsby, 1982). Protein flocculates are present in different sizes and shapes. It is noticeable that the size of the protein bodies in these aggregates is smaller (0.6 - 1.7 μm) than in the almond. It suggests a chemical interaction between lipid-proteins in the product, which changes the tertiary protein structure, reducing its volume (Kinsella, 1976; Voutsinas and Nakai, 1983). This fact implies a fat binding action that contributes to decreased oil release in the final product. Oil release is a characteristic property of the turrón, as free-oil inside the holes flows easily to the surface, producing a greasy appearance. If protein-lipid aggregates are destroyed in the manufacturing process, the product will probably be greasier, because of the higher free oil content. In this sense, kinetic and thermal energy supplied during the boixet step must be controlled, in order to improve the inversion system without breaking the protein association.

In conclusion, turrón structure can be described (Fig. 5) as an amorphous and porous matrix of sugar (80° Brix concentrated syrup) linked to ovalbumin, infiltrated with almond oil, which traps almond globular aggregates. These aggregates bind part of its fat content. Other almond cell fragments remain also in the sugar matrix, but no functional properties have been assigned to them. This structure arrangement in the product is in line with the hypothesis about the physical changes during the "boixet" process step.

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References


Discussion with Reviewers

D.G. Pechak: "Important change in texture and solid like mass... when cold". Is it the process that occurs in the boixet at 70-80°C or is it a texture change when cold?

Authors: The product entering the "boixet" is a viscous suspension but its consistency increases suddenly because of the heating and stirring treatment at the end of the step. When it gets cool, it turns into a kind of solid product.
Figure 5. Schematic representation of the role of the major components on the structure change of "Xixona turron" during the "boixet" step.

D.G. Pechak: "Oil bodies: 0.2-0.5 μm in diameter". Is this data from your work or the literature?

Authors: Oil bodies of toasted almond samples are not seen in our micrographs; before the SEM examination they might have been removed by fixative solutions and solvents. The fact that the almonds were toasted also facilitates oil extraction, as almond structure is partially destroyed. However TEM studies show the mentioned arrangement of spherosomes for oil bodies in some oil seeds (Aguilera and Stanley, 1990).

D.F. Lewis: What is the value for the sugar glass transition, Tg, for the mixture of sugars used in "Xixona turron"?

Authors: Tg values for the "Xixona turron" change with the addition of the different components during the manufacturing process. At the end of the concentration step (Fig. 1), and before ovalbumin addition, Tg value was 0 °C; however afterwards Tg increases to 16 °C, probably due to the protein binding. The mixture and crushing process with toasted almonds has a plasticizing effect over thermal product properties as Tg value becomes 12 °C in the final product (Fernández-Martín F, Galotto MJ, Chiralt A: Estudio calorimetrico del turron de Xixona, materias primas, productos intermedios y producto final. In: Proceedings of the III World Congress of Food Technology. Barcelona, Spain, 1991).
D.M. Manning: What is the influence of varying amounts of sugar, fat, protein and moisture on the texture and structure of the product?

Authors: Product moisture content remains practically constant during the "boixet" step as the sample has only been heated progressively up to 90 °C, however the product is manufactured with three different quality levels where the ratio of syrup to toasted almonds as well as the moisture content are different. For the lowest quality level, the ratio of water to protein is higher and the diffusion of almond proteins in the aqueous phase of syrup could be higher with a decrease in protein aggregates in the oil phase. Since so far we have been working only with the best quality product, it has not been studied as yet.

A textural characterization of commercial product with the different quality levels has been made, from various textural parameters. No correlation between these parameters and the level of the different components has been found. It seems that the size distribution of pores and holes where oil is lodged is probably the most important factor to define the textural characteristics of the final product. This distribution is strongly affected by the conditions during the "boixet" step (rate and type of stirring, time and temperature) and also by the uniformity of the molding process during the rest of the product.

D.M. Manning: What type of interaction occurs between the protein body and oils?

Authors: Different types of protein-lipid interactions, as a function of the polarity of its structure, have been described (Ericson B. Lipid Protein Interactions. In: Food Emulsions, Larsson K, Friberg SE (eds.), Marcel Dekker, Inc., New York) but we have not found specific studies for protein-lipids of almond oil. Nevertheless, as triglycerides are the major components of almonds and primary structure of proteins are strongly hydrophilic, it could be thought that hydrophobic associations occur, decreasing electrostatic repulsion, c.f., micelle formation where tertiary protein structure was very different from the native one.

D.M. Manning: Can you hypothesize any additional reasons why protein body size would change during heating?

Authors: Protein bodies observed in the final structure of "Xixona turron" are probably protein substances without an interfacial role and these have rested in a continuous oil phase as micellar aggregates in which structural arrangement of molecular segments is optimal to decrease the repulsive forces. As polar amino acids, e.g., aspartic and glutamic acids, are the major components of almond protein, probably an increase in the hydrophobicity of protein tertiary structure would imply a great folding of the macromolecular structure with a great decrease in its mean diameter.

D.F. Lewis: How do you distinguish between increased lipid binding which is simply due to the size reduction of protein aggregates (which could result from mechanical action) and that which is due to a specific chemical interaction between the proteins and lipids in the system?

Authors: It is quite difficult to distinguish between lipid binding due to physical changes from that due to chemical interactions. It appears true that there is a reduction in the size of protein bodies in the final product when they are compared with native structures in toasted almonds. We hypothesized in the last question that chemical hydrophobic interactions with lipids of almond oil could be responsible of the mean diameter decay. Nevertheless mechanical action also could result in a size reduction of protein aggregates; but in both cases chemical interaction are probably present. Isolation of proteins in almond and turron, and also the distribution of molecular weight analysis, could inform about its reduction in size during manufacturing process. TEM studies, including cryo-fracture techniques, could also support more precise data about the aggregation state of protein bodies in each product.

D.F. Lewis: How would you distinguish small starch grains from protein bodies in the SEM?

Authors: Chemical analyses of almond starch content showed no significant amount of this component (0.57 %) (Riquelme F. Características de almendras murcianas y su estabilidad. Doctoral Thesis. Universidad Politécnica de Valencia, Spain, 1982). On the other hand, light microscopic observation of almonds dyed with lugol (I2/I2 solution) did not allow us to see starch granules, so that it can be thought that spherical bodies observed in SEM preparation are proteins for the most part.

D.M. Manning: What is the function of ovalbumin?

Authors: Ovalbumin is mixed with syrup as a bleaching agent, but probably due to its interactions with sugar molecules, the melting characteristics (with a displacement of the glass transition temperature, Tg) and the viscosity of the product syrup change. The competitive water binding of ovalbumin and sugars in the syrup is probably the factor responsible for the changes observed in the syrup.