Influence of processing on the volatile profile of strawberry spreads made with isomaltulose

Peinado, I.; Rosa, E.; Heredia, A.; Escriche, I.* & Andrés, A.

Institute of Food Engineering for Development, Universitat Politècnica de València, P.O. Box 46022 Valencia, Spain

* Corresponding author. Tel.: +34 963877366; fax: +34 963877369

E-mail address: iescrich@tal.upv.es

Abstract

A new strawberry spread formulated with fructose and isomaltulose (replacing sucrose partially or totally) and a high percentage of fruit was developed in line with the new trend of healthier products. This work analyses the influence of some process variables (percentage of sugar, pectin and citric acid, and time of thermal treatment) on the volatile profile of these spreads with different formulations. The ripeness of the raw strawberries influences the concentrations of some of the compounds in the spreads, such as isobutyl acetate, butyl butyrate, 3-hexen-1-yl acetate or propan-2-ol. The process conditions have an important effect on the volatile profiles. Most of the esters and alcohols suffer a decrease whereas 13 new compounds appear, mostly furans (furfural, 2-acetylfurane, 5-methyl furfural, mesifurane) and aldehydes (octanal, nonanal, decanal and benzaldeyhde). In general, the spreads formulated with sucrose-isomaltulose, containing higher levels of pectin and citric acid gave better results in the preservation of the original aromatic compounds in raw strawberries.

Keywords: strawberry, healthy fruit spread, isomaltulose, volatile profile, aroma

1. Introduction

Recently, the population of developed countries has modified its nutritional habits as a consequence of new life styles. In fact, many studies have reported that the new eating habits related to this life style are causing health problems. An example is the relationship established between fast food with obesity and diabetes 2 (Pereira, Kartashov, Ebbeling, Van Horn, Slattery, Jacobs & Ludwig, 2005; Jeffery, Baxter, Mcguire & Linde, 2006; Frank & Vasanti, 2010; Fraser & Edwards, 2010; McPhail, Chapmanb & Beagan, 2011). From this point of view, the development of new products such as fruit spreads formulated with healthier sugars like fructose and isomaltulose would be interesting for certain groups of the population such as children and senior citizens. These products should not have undesirable effects like caries and diabetes (related with sugar consumption), and therefore would be more appropriate for these niches of the population than traditional jams.

Fruit is a food group which is receiving more attention among the population due to its interesting and healthy properties such as high functional and nutritional value, being rich in fibre, minerals, vitamins, terpens antioxidant compounds, etc.(Gillman, Cupples, Gagnon, Posner, Ellison, Castelli & Wolf, 1995; Cavanah, Hipwell & Wilkinson, 2003; Dhiraj, Vattem & Shetty, 2005). Among the wide variety of available fruit, one of the most valued is the strawberry, not only because of its high content in vitamins and minerals, but also because of its organoleptic characteristics such as taste and aroma. Strawberries are rich in vitamin C, sometimes in an even higher concentration than oranges. They are also rich in minerals (iron, iodine, calcium, phosphorus, magnesium and potassium (Ávila, Beltrán, Cuadrado, Del Pozo, Rodríguez & Ruiz, 2009) and more than 33 identified organic acids, for example: citric, malic, oxalic, and folic, (Rizzolo, Lombardi, Lovati, Tagliabue & Testoni 1995; Forney, Kalt, McDonald, & Jordan, 1996;

Azodanlau, Darbellay, Luisier, Villettaz, & Amado, 2003, 2004). Strawberries are very problematic for industrial processing as they are seasonal, and have a high water content which makes them very perishable. Besides, strawberry has healthy, sensory characteristics which make it a very attractive fruit for processing. Therefore, the food industry has an increased interest in developing new kinds of processed fruit products whose sensorial characteristics are not very different to the fresh fruit. This could be the case of strawberry spreads, as they present some characteristics similar to fresh fruit but on the other hand are more stable than the fresh product since the a_w and the moisture of the product are reduced. The big difference between a fruit spread and a jam is that in a fruit spread cooking to reach a final soluble solid content is avoided, as it provokes the greatest changes from a nutritional, sensorial and functional point of view. Moreover, a jam must have at least 45 ºBrix, whereas a spread fruit does not have any restriction related to sugar content (BOE 04/07/07; RD: 863/2003). Additionally, these spreadable products might be considered healthier when the sucrose is replaced by other sweeteners such as isomaltulose, a sugar especially indicated for children and senior citizens as it does not produce caries, and moreover is slowly released in the blood (Matsuyama, Sato & Hoshino, 1997; Schiweck, Munir, Rapp, Schenider, & Bogel, 2000; Lina, Jonker & Kozianowski, 2002).

The food industry is more and more concerned with the elaboration of healthier food products without forgetting the importance of taste and flavour, since they are very important characteristics from the consumers' point of view. These attributes which can be quantified in terms of the volatile profile are important for both the fresh and the processed product. In the case of strawberries, more than 360 volatile compounds have been identified, including esters and acids, together with alcohols, ketones, lactones and aldehydes in minor quantities (Larsen, Poll & Olsen, 1992; Forney, 2001). Esters, which are responsible for the fruity, floral aroma make up more than 25% of the total mass of the volatiles in this ripe fruit (Maarse, 1991; Gomes da Silva *&* Chaves das Neves, 1999; Forney, 2001). Aldehydes and furanones also play important roles in strawberry aroma (Forney, 2001; Bood & Zabetakis, 2002), as well as Terpenic and sulfuric compounds, which have an important impact on the characteristic aroma of red berries even though they only represent a small portion of the volatile compounds (Dirinck, De Pooter, Willaert & Schamp 1981; Azodanlau, *et al*., 2003, 2004).

The aim of this work was to analyze the influence of the different process variables (wt % of isomaltulose, wt % of pectin, wt % of citric acid and time of heat treatment) on the volatile profile of different strawberry fruit spreads made using healthier sugars such as isomaltulose or fructose to replace sucrose partially or totally.

2. Material and Methods

2.1. Raw material

Fifteen batches of raw strawberries (*Fragaria vesca,* Camarosa) acquired in a local supermarket, where used for the present work. The experimental work was performed between February and June of 2010. Each batch of strawberries was sorted in order to eliminate damaged fruit and group the samples in terms of colour, shape and ripeness. Then they were cut and washed in chlorinated water to eliminate possible field residues.

2.2. Methodology

Formulation of the spreadable products:

Following the surface-response methodology, a statistical central composite design 2^4 + start (Gómez & Gómez, 1984; Kaur, Wani, Oberoi & Sogi., 2008) was applied to analyze the influence of four independent variables X_1 (% of isomaltulose), X_2 (% of pectin), X_3 (% of citric acid) and X_4 (time of heat treatment) on the volatile profile of spreadable strawberry products (table 1). Two kinds of strawberry spread were formulated following two identical replicates of the design. One group of samples containing sucrose and isomaltulose (as sucrose was considered to be the reference sugar) and a second group formulated with fructose and isomaltulose (both considered healthy sugars). The target concentration of the final product was 50 ºBrix, therefore the sweetness of the sugars was not a determinant parameter when the different mixtures where made to formulate the products. These mixtures between two sugars were needed since the solubility of isomaltulose does not permit the required final sugar concentration of 50 ºBrix (Kaga & Mizutani, 1985; Schiweck *et al*., 2000).

The formulation of the products was the result of the implementation of dry osmotic dehydration studied previously (Peinado, Rosa, Heredia & Andrés*,* 2008, Rosa, Peinado, Heredia & Andrés*,* 2008). In this case, the product was directly formulated by mixing the ingredients in the correct proportions to reach the established concentration of 50 oBrix, therefore, avoiding the typical equilibrium stage which takes place during osmotic dehydration. The amounts of raw strawberry and sugar needed were calculated according to the correspondent mass balance and they were dependent on the soluble solids content in the raw strawberries. The ingredients in the spreadable strawberry products were: strawberries, sugars (sucrose or fructose, and isomaltulose), pectin (as a gelling agent), potassium sorbate at a fixed concentration of 500 ppm (as a microbiological preserver) (Karabulut, Lurie & Droby, 2001; Castelló, Fito & Chiralt, 2006) and citric acid (as a colour preserver). Once the ingredients were mixed, the product was heated until it reached 85 ºC. This temperature was necessary to make "hot canning" effective, as well as to allow the pectin to dissolve and then gel, so that the gel structure would not break up during storage. Then, the product was placed in glass jars and some of the samples, depending on the statistical design, were heated for 5, 10, 15 or 20 minutes in a bath of boiling water. Finally, they were stored at room temperature for 24 hours before the correspondent analyses were performed.

2.3. Physicochemical analyses

All the physicochemical analyses were carried out on raw strawberry puree, and the final spreads. All measurements were carried out in triplicate.

Moisture content was determined gravimetrically by drying to a constant weight in a vacuum oven at 60 ºC (method 20.103 AOAC, 1980). Soluble solids content (ºBrix) was measured with a refractometer at 20 ºC (ATAGO 3 T). For the strawberry spread, dilution at a ratio of 4 g water for each gram of sample was necessary. Water activity (a_w) was determined with a dew point hygrometer (FA-st lab, GBX) and pH was determined with a pH-meter (SevenEasy, Mettler Toledo).

2.4. Volatile compound analysis

Aromatic compounds were extracted by purge and trap thermal desorption; (Torres, Chiralt & Escriche, 2012) 20 g of raw strawberry or strawberry spread spiked with 200 µL 2-pentanol (10 µg/mL as an internal standard), were placed in a purging vessel flask and left in a water bath at 45 ºC for 20 min. During this time, purified nitrogen (150 mL min^{-1}) was forced through a porous filter placed at the bottom of the vessel, producing a stream of bubbles which passed through the sample drawing the volatile compounds. These were trapped in a 100 mg porous polymer (Tenax TA, 20–35 mesh) packed into a glass tube placed at the end of the system. A total of 3 extracts were obtained for each sample. The volatile compounds were subsequently thermally desorbed using a direct thermal desorber (TurboMatrix TD, Perkin ElmerTM, CT-USA). Desorption was performed under a 10 mL min^{-1} helium flow at 240 ºC for 10 min. The volatiles were then cryofocused in a cold trap at -30 ºC and transferred directly onto the head of the capillary column by heating the cold trap to 250 ºC (at a rate of 99 ºC/s).

GC–MS analyses were performed using a Finnigan TRACETM MS (TermoQuest, Austin, USA). Volatile compounds were separated using a BP-20 capillary column (SGE, Australia) (60 m length, 0.32 mm i.d., 1.0 lm film thickness). Helium at a constant flow rate of 1 mL min^{-1} was used as a carrier gas. The temperature was programmed to increase from 40 ºC (2 min hold time) to 190 ºC at 4 ºC min^{-1} and finally to 230 ºC at 10 ºC min^{-1}. The MS interface and source temperatures were 250 ºC and 200 ºC, respectively. Electron impact mass spectra were recorded in impact ionization mode at 70 eV and with a mass range of m/z 33–433. A total of three extracts were obtained for each sample.

The volatile compounds were tentatively identified by comparing their mass spectra (*m/z* values of the most important ions) with spectral data from the National Institute of Standards and Technology 2002 library as well as retention indices published in the literature (Kondjoyan & Berdagué, 1996; pherobase.com). Relative retention indices were determined by injection into the Tenax of a solution containing the homogenous series of normal alkanes (C_8 –$C_{20;}$ by Fluka Buchs, Schwiez, Switzerland) in the same temperature-programmed run, as described above. Semiquantitative analyses were carried out (Soria, Martínez-Castro & Sanz, 2008). Quantification was not considered necessary since the objective of this study was to evaluate the differences between the different treatments considering all of the identified compounds without being limited to those for which standards were available.

The data (µg/100 g of fresh strawberry) were expressed using the amount of internal standard and the relative area of each compound with respect to that of the internal standard, assuming a response factor equal to one.

2.5. Statistical analysis

As it has already been mentioned, a central composite design was applied to analyze the influence of four independent variables X_1 (% of isomaltulose), X_2 (% of pectin), X_3 (% of citric acid) and X_4 (time of thermal treatment) at five levels, on the aromatic profile of the strawberry spreads.

The statistical analyses of variance (ANOVA) with a confidence level of 95% (p-value ≤ 0.05) were carried out by means of the software package Statgraphics Plus 5.1 to estimate the significant differences between the raw strawberry batches used. Furthermore, Principal Component Analysis, PCA, (Martens & Næs, 1989) (Unscrambler version 10.X; CAMO Process AS, Oslo, Norway) was applied to describe the relationships between the volatile compounds and process variables (wt % of isomaltulose, wt % of pectin, wt % of citric acid ant time of heat treatment).

3. Results and discussion

3.1. Influence of formulation on the physicochemical parameters of spreadable strawberry products

In order to evaluate the natural variability of physicochemical parameters in the raw batches due to their different levels of ripening, several analyses of variance (ANOVA) were performed. Table 2 shows the mean values and the standard deviation of the physicochemical parameters (water activity (a_w), moisture content (x^w), soluble solids content (x^{ss}), and pH) as well as the F- ratio obtained from the simple ANOVAs performed. The results showed significant differences between the different raw batches, which should be taken into account when formulating the product.

After processing, all the strawberry spreads showed a very similar composition in terms of soluble solids (x^{ss}) and moisture (x^w) content as they were formulated in order to reach a final concentration of 50 ºBrix. Table 2 shows the water activity (a_w) and pH values of the strawberry spreads for all the combinations of the different variables to obtain 26 spreads with sucrose-isomaltulose and 26 products with fructose-isomaltulose. The pH varied for the spreads, their values ranging between 3.03 ± 0.42, for the spreads which had the lowest percentage of citric acid, and 2.80 ± 0.03 for the spreads with the highest percentage of citric acid. These differences could have a direct influence on the stability and acceptation of the product.

3.2. Influence of raw material and processing on the volatile profile of spreadable strawberry products

29 volatile compounds were tentatively identified and semi-quantified in the volatile profile of raw strawberries. Table 3 shows the relative retention index (RI) calculated for each volatile compound, their concentration in the different raw batches expressed as C_F (μg of volatile compound / 100 g raw strawberry), as well as the statistical results of the simple analysis of variance performed for each compound. These compounds belong to four different chemical families: 16 esters, 10 alcohols, 2 aldehydes and 1 ketone. Specifically, ethyl hexanoate and methyl hexanoate (fresh fruity fragrance), methyl octanoate (orange wine aroma) and ethyl octanoate (aquavit aroma) are some of the main contributors to the strawberry volatile profile, which is consistent with previous studies (Perez et al. 1992, 1997; Zhang et al., 2009). Some aldehydes, such as hexenal, are also responsible for the typical green notes in this fruit. It is well know that amino acids, sugars and lipids are precursors of aroma compounds of esters. In this pathway, the bioconversion of the amino acids, sugars and lipids into acids, aldehydes or alcohols, and consequently into esters are catalyzed by two key enzymes: alcohol acyltransferases and alcohol dehydrogenases (Charles, 2000; Zhang et al., 2009).

As for the physicochemical properties, the statistical analysis of variance (ANOVA) showed significant differences between the different batches of raw strawberries due to the different concentrations of the volatile compounds. These differences in the volatile profile are usually a consequence of the natural variability in this fruit (Larsen *et al*., 1992). Compounds such a hexanal, methyl butyrate, ethyl butyrate, butyl acetate, ethyl hexanoate, 2- hexenyl and 3-hexenyl acetate, vary their concentration depending on the level of ripeness (Azodanlou *et al*., 2004). Specifically in this work, there were no differences in the butyl acetate concentration between the different raw batches, while huge differences in the 3-hexenyl acetate concentration were observed (table 3).

Some authors have pointed out that the concentration of furaneol (2,5-dimetyl-4-hydroxi-furane), a compound which has an important contribution to the strawberry volatile profile (Sanz, Richardson & Pérez, 1995; Ulrich, Rapp & Hoberg, 1995; Schieberle & Hofmann, 1997; Zabetakis & Holden, 1997), is very variable depending on the variety and level of ripeness of the strawberries (Pérez, Olías, Sanz & Olías, 1996; Pérez, Olías, Olías & Sanz, 1999). However, it was not detected in this study, probably because the extraction method did not reach temperatures above 45 ºC which are required to extract some compounds such as furaneol (Escriche, Chiralt, Moreno & Serra, 2000).

Table 3 shows the mean values of the maximum and minimum net variations of the volatile compounds originally detected in the raw strawberries, and detected again in all 52 strawberry spreads (26 sucrose-isomaltulose spreads and 26 fructose-isomaltulose spreads) after processing. In general terms, the volatile profile was modified with respect to the raw strawberry profile. The concentration of the majority of the volatile compounds was modified, according to the ratio $((C_0\text{-}C_F)/C_F)$, C_0 being the concentration of the volatile compound (VC) in the strawberry spread expressed as μg de VC / 100 g of raw strawberry, and C_F the initial concentration of this compound in its correspondent raw batch. Some steps which take place during the processing of spreadable products, e.g. cutting or blending, sugar addition, etc., lead to an increase in the activity of enzymes responsible for the development of aroma. This fact has been reported when different kinds of fruit, including strawberries, are submitted to particular stress factors, such as osmotic stress, UV light, a change in pH or contact with metal ions (Pérez, Sanz, Rios & Olias, 1993; Zabetakis & Holden, 1997; Escriche et al., 2000).

In the present work, some original compounds in fresh strawberries such as ethyl propionate, ethyl hexanoate, 3-hexen-1-yl acetate or 3-hexen-1-ol, were not identified in the strawberry spreads, whereas 13 new compounds were detected after processing. These new compounds were classified as 6 aldehydes, 1 alcohol, 4 furans, 1 terpene, and 1 nitrile as it can be seen in table 4 which shows the concentration of these new compounds expressed as μg de VC / 100 g of spread (net variation cannot be calculated as the initial concentration in the raw batches would be equal to 0).

It has to be mentioned that of the compounds whose concentration changed after processing, the esters and alcohols decreased their concentration in most of the spreads, except hexyl acetate, ethanol and pentan-2-ol which increased, specifically the last one. The apparition of other compounds such as eucalyptol in the formulated products should be highlighted as well. Some authors have confirmed the liberation of alcohols during the heat treatment of fruit juices as a consequence of the hydrolysis of their corresponding glycoside precursors (Barron & Etievant, 1990).

For the aldehydes, hexenal and 2-hexenal increased their concentration and 6 new aldehydes were also found. This generation of new compounds may be due to the biooxidation of the lipids, mostly non-saturated fatty acids, as a consequence of the cell wall disruption (Yilmaz & Oxylipin, 2001).

On the other hand, the activation of Maillard reactions, or the catabolism of carotenoids and non-saturated fatty acids promote the generation of volatile compounds related to a cooked flavour (Servili, Selvaggini, Taticchi, Begliomini & Montedoro*,* 2000). In this sense, furfural is the most representative aroma generated by Maillard reactions, although it can also originate due to the degradation of ascorbic acid (Barron & Etievant, 1990). It is important to note that the concentration of furfural in spreadable products was higher in those formulated with fructose-isomaltulose. This could be due to the presence of fructose (a reducing sugar) and the precursors of these reactions, in this formulation. It is well-known that the type of sugar influences both the kind and concentration of the generated flavour compounds during Maillard's Reactions (Fisher & Scott, 1997).

A PCA was conducted to evaluate the global effect of processing on the volatile fraction of the spreadable products, from a descriptive point of view. Figure 1 shows the sample scores of the different raw strawberry batches (A-O) and the strawberry spreads (S1 to S26, and F1 to F26), as well as the compound loadings of the PCA analysis performed. The first two components explain 47 % of total variance (PC1 33 % and PC2 14 %). In the score plot, proximity between samples indicates similar behaviour in terms of the volatile profile. In the loading plot, proximity between aromas demonstrates some similarity in their concentration levels.

It can be seen that the different raw batches are located on the left, while the formulated products (S1 to S26, and F1 to F26), independent of the sugar mix used (sucrose-isomaltulose or fructose-isomaltulose), are distributed on the right. Moreover, the raw batches are separated depending on their volatile composition, so those used during the initial months of the experiments (A-E) are placed in the left of this group, while the other raw batches (F-O), with a high level of ripeness, are placed on the right of this group. This fact confirms that the heterogeneity between the different batches could be due to different levels of ripeness as was mentioned in the characterization of the raw material. On the other hand, the distribution of the strawberry spreads confirms what was mentioned before; some characteristic compounds of raw strawberry, such as ethyl acetate and some alcohols are placed on the left side as they are more related to raw strawberries. On the opposite, compounds generated after processing such as aldehydes and furans are placed on the right side with the strawberry spreads.

Once it was clear that the volatile composition of the raw material had an important effect on the volatile profile of the formulated spreads, it was important to evaluate the effect of the different process variables, as well. Thus new PCA analyses were performed, but this time sucrose-isomaltulose spreads were considered separately from fructose-isomaltulose spreads. Moreover, in order to avoid the variation due to the differences between batches, the net mass variations were used to analyze the original volatile compounds in raw strawberries whose concentration was modified as a consequence of processing. Finally, PCAs of generated compounds were also performed.

Figure 2 shows the PCA results of the different sucrose-isomaltulose spreads (2a) and fructose-isomaltulose spreads (2b), as well as the volatile compounds whose concentration was modified. While figure 3 shows the PCA results for the volatile compounds generated as a consequence of the processing expressed as μg de VC/ 100 g of strawberry spread.

In these figures, it can be seen that the majority of products are located together, meaning no huge differences in their volatile composition. However, for the modified volatile compounds in sucrose spreadable products, formulations S-19, S-20, S-25 and S-26, are separated from the others, meaning a minor evolution of their volatile profile compared with fresh fruit. Moreover, for the volatile compounds generated, in addition to those formulations, formulations S-1, S-2, S-3, S-4, S-21 and S-22 were also separated from the rest. These products happened to be the ones with higher levels of pectin and citric acid, which may suggest a major retention of the original volatile compounds with high levels of these two variables. In fact, some authors have confirmed the hardening of the three-dimensional net formed by pectin-fruit-sugar when the levels of citric acid and pectin are increased (Rauch, 1987; Guichard, 1996; Dervisi, Lamb & Zabetakis, 2001).

On the other hand, the products formulated with fructose, showed different behaviour. In this case, the formulations were grouped closer together than the sucrose spreadable products, which could be due to a major homogeneity between products. Furthermore, only formulations F-1 and F-2 were separated from the rest in the graphs for the modified compounds and the generated compounds. These results would suggest a different behaviour of the food matrix depending on the sugar added, those products formulated with sucrose and higher levels of pectin and citric acid being the ones which showed a better retention of the original volatile compounds and a minor evolution compared to raw strawberry.

4. Conclusions

The volatile profile of spreadable strawberry products was influenced by the ripeness of the raw material as well as by processing conditions. The percentages of pectin and citric acid were the variables which had most influence on the retention of the typical volatile profile of fresh strawberries, especially in the products formulated with sucrose and isomaltulose. Most of the esters and alcohols identified in raw material decreased; whereas new compounds, mostly furans and aldehydes, were generated as a consequence of processing. In future studies, it would be interesting to analyze the volatile profile changes as a consequence of storage in order to accurately establish the optimal shelf-life of the final product together with physicochemical and microbiological analysis.

Acknowledgments

Authors would like to thank Dirección General de Investigación del Ministerio de Ciencia y Tecnología (AGL2008-01745/ALI) as well as the Universitat Politècnica de València for the financial support given to this investigation.

References

Ávila, J. M., Beltrán, B., Cuadrado, C., Del Pozo, S., Rodríguez, M. V. & Ruiz, E. (2009). La Alimentación Española: características nutricionales de los principales alimentos de nuestra dieta. Ministerio de Agricultura, Pesca y Alimentación. Fundación Española de la Nutrición. España. 632 pp.

Azodanlau, R., Darbellay, C., Luisier, J. L., Villettaz, J. C., & Amado, R. (2003). Quality assessment of strawberries (*Fragaria* species). *Journal of Agricultural and Food Chemistry, 51*, 715–721

Azodanlau, R., Darbellay, C., Luisier, J.L., Villettaz, J.C. & Amado, R. (2004). Changes in flavour and texture during the ripening of strawberries. *Eur Food Res Technol, 218:*167–172.

Barron, D. & Etievant, P. X. (1990). The volatile constituents of strawberry jam. *Z Lebensm Unters Forsch. 191,* 275-289.

B.O.E. 04/07/07. Real Decreto 863/2003. Norma de calidad para la elaboración, comercialización y venta de confituras, jaleas, mermeladas de frutas y cremas de castañas.

Bood, K. G., & Zabetakis, I. (2002). The biosynthesis of strawberry flavor (II): Biosynthetic and molecular biology studies. *Journal of Food Science, 67*: 2–8.

Castelló, M.L., Fito, P.J. & Chiralt, A. (2006). Effect of osmotic dehydration and vacuum impregnation on respiration rate of cut strawberries. *LWT 39,* 1171–1179.

Cavanah, H.M., Hipwell, M. & Wilkinson J.M. (2003). Antibacterial activity of berry fruits used for culinary purposes. *Med Food*; 6 (1): 57-61.

Charles F, Willy K, Michael J. 2000. The composition of strawberry aroma is influenced by cultivar, maturity, and storage. HortScience, 35, 1022-1026.

Dervisi, P., Lamb, J. & Zabetakis, I. (2001) High pressure processing in jam manufacture: effects on textural and colour properties. (2001). *Food Chemistry Vol 73, (1)*, 85-91.

Dhiraj, A., Vattem , R.G. & Shetty, K. (2005). Enhancing health benefits of berries through phenolic antioxidant enrichment: focus on cranberry. *Asia Pac J Clin Nutr*. 14 (2):120-130.

Dirinck, P.J., De Pooter, H.L., Willaert, G.A. & Schamp, N.M. (1981). Flavor quality of cultivated strawberries: the role of the sulfur compounds. *J Agric Food Chem* 29 (2):316-321.

Escriche, I., Chiralt, A., Moreno, J. & Serra, J. (2000). Influence of Blanching-osmotic Dehydration Treatments on Volatile Fraction of Strawberries. *Journal of Food Science, V 65, (7),* 1107–1111.

Fisher, C. and Scott, T.R. (1997). Chapter 2: Food Flavours. In: Food Flavours. Biology and Chemistry. The Royal Society of Chemistry, Thomas Graham House. Cambridge, England.

Forney, C. F., Kalt, W., McDonald, J. E., & Jordan, M. A. (1996). Changes in strawberry fruit quality during ripening on and off the plant. Acta Horticulturae, 464, 506.

Forney, C. F. (2001). Horticultural and other factors affecting aroma volatile composition of small fruit. *HortTechnology 11*,529-538.

Frank, B. H. & Vasanti S. M. (2010). Sugar-sweetened beverages and risk of obesity and type 2 diabetes: Epidemiologic evidence. *Physiology & Behavior 100,* 47–54.

Fraser, L.K. & Edwards, K.L. (2010). The association between the geography of fast food outlets and childhood obesity rates in Leeds, UK. *Health & Place 16*, 1124–1128.

Gillman, M.W., Cupples, L., Gagnon, D., Posner, B., Ellison, R., Castelli, W.P. & Wolf, P.A. (1995). Protective Effect of fruits and vegetables on development of stroke in Men. *J Am Med Assoc*; 273: 1113-1117.

Gomes da Silva, M.D.R. & Chaves das Neves, H.J. (1999). Complementary use of hyphenated purge and trap gas chromatography techniques and sensory analysis in the aroma profiling of strawberries (Fragaria ananassa). *J Agric Food Chem 47*,4568-4573.

Gómez, K.C. & Gómez, A.A. (1984). *Statistical procedures for agricultural research*. (2nd ed). New York: Jhon Wiley and sons.

Guichard, E. (1996) Interactions Between Pectins and Flavor Compounds in Strawberry Jam. *American Chemical Society Symposium Series*. *633* (11), 118–129.

Jeffery, R., Baxter, J., Mcguire, M. & Linde, J. (2006). Are fast food restaurants an environmental risk factor for obesity?. *International Journal of Behavioral Nutrition and Physical Activity* 3(2): 2006.

Kaga, T. y Mizutani, T. (1985). "Verwendung von Palatinose fur Nahrungsmittel. *Seito Gijutsu Kenkyukaishi*" *34*, 45-57.

Karabulut, O. A., Lurie, S. & Droby, S. (2001). Evaluation of the use of sodium bicarbonate, potassium sorbate and yeast antagonist for decreasing posthavest decay of sweet cherries. *Postharvest Biology and Technology, 23*, 233–236.

Kaur, D., Wani, A., Oberoi, D.P.S. & Sogi, D.S. (2008). Effect of extraction conditionson lycopene extractions from tomato processing waste sking using response surface methodology. *Food Chemestry 108*, 711-718.

Kondjoyan, N. & Berdagué, J. L. (1996). A Compilation of Relative Retention Indices for de Analysis of Aromatic Compounds. Ed. Du laboratoire Flaveur. 1st edition.

Larsen, M., Poll L. & Olsen, C.E. (1992). *Z Lebensm Unters Forsch* 195:536–539.

Lina, B.R., Jonker, G. & Kozianowski, G. (2002). Isomaltulose (Palatinose Re review of biological and toxicologycal studies). *Food and Chemical Toxicology, 40*, 1375-1381.

Maarse, H (ed) (1991) Volatile compounds in foods and beverages. *Dekker, New York.*

Martens, H., & Naes, T. (1989). *Multivariate calibration. Chichester, Wiley. UK.*

Matsuyama, J., Sato, T. & Hoshino, E. (1997). Acid production from palatinose, palatinit, erythritol and maltitol by bacteria isolated from dental plaque on human deciduous teeth. *Japanese Journal of Oral Biology*, *39*, 91-99.

McPhail, D., Chapmanb, G. E. & Beagan, B. L. (2011). "Too much of that stuff can't be good": Canadian teens, morality, and fast food consumption. *Social Science & Medicine 73,* 301-307.

Peinado, I., Rosa, E., Heredia, A. & Andrés, A. (2008). Estudio comparativo del color en fresa deshidratada osmóticamente con sacarosa, sorbitol e isomaltulosa. Póster-Artículo, Congreso Iberoamericano sobre Seguridad Alimentaria. V Congreso Español de Ingeniería de Alimentos. Book of Abstracts, p. 165.

Pereira, M., Kartashov, A., Ebbeling, C., Van Horn, L., Slattery, M., Jacobs, D., Ludwig, D. (2005). Fast-food habits, weight gain and insulin resistance (the CARDIA study): 15-year prospective analysis. *The lancet*, 365:36-42.

Pérez, A. G., Olías, R, Olías, J.M. & Sanz, C. (1999). Biosyntesis of 4-Hydroxy-2,5-dimethyl- 3(2H)-furanone and Derivatives in Vitro Grown Strawberries. *J. Agric. Food Chem. 47*: 655- 658.

Pérez, A.G., Olías, R., Sanz. C. & Olías, J.M. (1996*).* Furanones in Strawberries: Evolution during Ripening and Postharvest Shelf Life. *J. Agric. Food Chem. 44*: 3620-3624.

Pérez, A. G., Sanz, C., Rios, J. J., y Olias, J. M. (1993). Estudio comparativo de los perfiles aromáticos de manzana, plátano y fresa. *Revista Española de Ciencia y Tecnología de Alimentos, 33,* 665–677.

Rauch, G.H. (1987). Fabricación de mermeladas. Ed. Acribia, S.A. Zaragoza. España.

Rizzolo, A., Lombardi, P., Lovati, F., Tagliabue, S., & Testoni, A. (1995). Valutazione della componente aromatica dei frutti di fragola. *L'Informatore Agrario, LI(44*), 37–41.

Rosa, E., Peinado, I., Heredia, A. & Andrés, A. (2008). Deshidratación osmótica de frutas por vía seca. Una alternativa al uso de disoluciones. Póster-Artículo, Concgreso Iberoamericano sobre Seguridad Alimentaria. V Congreso Español de Ingeniería de Alimentos. Book of Abstracts, p. 168.

Sanz, C., Richardson, D.G. & Pérez, A.G. (1995). *2*,5-Dimethyl-4- hydroxy-3(2H) furanone and derivatives in strawberries during ripening. *ACS, Symp Ser 1995:*268–275.

Schieberle, P. & Hofmann, T. (1997). Evaluation of the Character Impact Odorants in Fresh Strawberry Juice by Quantitative Measurements and Sensory Studies on Model Mixtures. J. *Agric. Food Chem. 5*, 227-232.

Schiweck ,H., Munir, M., Rapp, K. M., Schenider, B. & Bogel, M. (2000). New developments in the use of sucrose as an industrial bulk chemical. *Zuckerindustrie 115*, 555-565.

Servili, M., Selvaggini, R., Taticchi, A., Begliomini A.L., & Montedoro, G.F. (2000). Relationships between the volatile compounds evaluated by solid phase microextraction and the thermal treatment of tomato juice: optimization of the blanching parameters. *Food Chemistry, 71,* 407-415.

Soria, A. C., Martínez-Castro, I. & Sanz, I. (2008). Some aspects of dynamic headspace analysis of volatile components in honey. *Food Research International*, *41*, 838-848.

Torres, J.D., Chiralt, A. & Escriche, E. (2012). Development of volatile fraction of fresh cut osmotically treated mango during cold storage. *Food Chemistry 130*, 921–927.

Ulrich, D., Rapp, A. & Hoberg, E. (1995). *Z Lebensm Unters Forsch 200*,217–220.

Yilmaz, E., & Oxylipin (2001). Pathway in the biosynthesis of fresh tomato volatiles. Turk. *J. Biol*. *25*, 351–360.

Zabetakis, I. & Holden, M.A. (1997). Strawberry Flavour: Analysis and Biosynthesis. *J. Sci. Food Agric., 74*, 424-434.

Zhang, Y.T., Wang, G.X., Dong, J., Zhong, C.F., Kong, J., Li, T.Z., Han, Z.H. (2009). Analysis of Volatile Components in Strawberry Cultivars Xingdu 1 and Xingdu 2 and Their Parents. *Agricultural Sciences in China, 8(4),* 441-446.

Grab, W. (1998). Fruit Flavours. In: Flavourings: Production, Composition, Applications, Regulations. Ed: Ziegler, E. & Ziegler, H. Wiley-WCH, Weinheim, Germany.

www.pherobase.com. Date of last view 3.08.2010.

Table 1. Independent variables and their level used for central composite design.

composite design.

Independent variables	Symbol[c]	Coded variable levels				
		-2	**-1**	**0**	**1**	**2**
Isomaltulose (%)[a]	X_1	0	12.5	25	37.5	50
Pectin (%)[b]	X_2	0.5	1	1.5	2	2.5
Citric acid (%)[b]	X_3	0	0.25	0.5	0.75	1
Heat treatment time (min)	X_4	0	5	10	15	20

a: % of isomaltulose in the total amount of sugar mix (sucrose-isomaltulose or fructose-isomaltulose).
b: in final product.
c: Symbol with which each independent variable is cited in the text.

Table 2. Physicochemical values (water activity (a_w) moisture content (x^w), soluble solids content (x^{ss}), and pH) and F- ratio of the different batches of strawberry. Combination of the different variables performed to obtain 26 products with sucrose and 26 products with fructose. Water activity (a_w) and pH of the spread products (n=3).

Physicochemical parameters of raw strawberry batches (ANOVA results)

	Mean		F-ratio
a_w	0.988	(0.003)	26.75***
x^w	0.925	(0.012)	1.04
x^{ss}	0.068	(0.006)	148.09***
pH	3.45	(0.08)	85.08***

Strawberry spreads

% S or % F	X_1 % I [a]	X_2	X_3	X_4	Treatments with Fructose: sample	a_w	pH	Treatments with Sucrose: sample	a_w	pH
62	37.5	0.75	2	15	**F-1**	0.917 (0.006)	2.947 (0.006)	**S-1**	0.913 (0.002)	2.980 (0.020)
62.5	37.5	0.75	2	5	**F-2**	0.915 (0.002)	2.930 (0.009)	**S-2**	0.926 (0.003)	2.910 (0.030)
75	25	1	1.5	10	**F-3**	0.905 (0.002)	2.810 (0.002)	**S-3**	0.917 (0.002)	2.820 (0.020)
62.5	37.5	0.75	1	5	**F-4**	0.905 (0.002)	2.897 (0.006)	**S-4**	0.922 (0,002)	2.903 (0.006)
75	25	0.5	1.5	0	**F-5**	0.911 (0.002)	3.003 (0.006)	**S-5**	0.931 (0.002)	3.037 (0.006)
62.5	37.5	0.75	1	15	**F-6**	0.912 (0.002)	2.890 (0.020)	**S-6**	0.927 (0.002)	2.890 (0.020)
87.5	12.5	0.75	1	15	**F-7**	0.895 (0.002)	2.900 (0.020)	**S-7**	0.906 (0.002)	2.910 (0.020)
100	0	0.5	1.5	10	**F-8**	0.896 (0.002)	2.977 (0.006)	**S-8**	0.922 (0.012)	3.020 (0.002)
75	25	0.5	0.5	10	**F-9**	0.903 (0.002)	2.990 (0.002)	**S-9**	0.934 (0.002)	3.017 (0.006)
87.5	12.5	0.75	1	5	**F-10**	0.912 (0.003)	2.797 (0.006)	**S-10**	0.934 (0.003)	2.867 (0.006)
75	25	0	1.5	10	**F-11**	0.907 (0.002)	3.390 (0.020)	**S-11**	0.929 (0.012)	3.440 (0.020)
75	25	0.5	1.5	10	**F-12**	0.910 (0,002)	2.990 (0.020)	**S-12**	0.916 (0.012)	3.023 (0.006)
87.5	12.5	0.25	1	15	**F-13**	0.900 (0.002)	3.180 (0.002)	**S-13**	0.921 (0.002)	3.230 (0.020)
75	25	0.5	1.5	10	**F-14**	0.905 (0.002)	3.010 (0.009)	**S-14**	0.93 (0.002)	3.030 (0.020)
62.5	37.5	0.25	1	5	**F-15**	0.911 (0.002)	3.130 (0.002)	**S-15**	0.926 (0.002)	3.200 (0.002)
87.5	12.5	0.25	1	5	**F-16**	0.894 (0.007)	3.183 (0.006)	**S-16**	0.924 (0.002)	3.200 (0.020)
62.5	37,5	0.25	1	15	**F-17**	0.910 (0.002)	3.167 (0.006)	**S-17**	0.924 (0.002)	3.153 (0.006)
87,5	12,5	0.25	2	5	**F-18**	0.909 (0.001)	3.163 (0.012)	**S-18**	0.919 (0.002)	3.160 (0.002)
75	25	0.5	2.5	10	**F-19**	0.906 (0.002)	2.917 (0.006)	**S-19**	0.922 (0.002)	3.007 (0.006)
87.5	12.5	0.75	2	15	**F-20**	0.902 (0.002)	2.873 (0.006)	**S-20**	0.907 (0.002)	2.873 (0.006)
87.5	12.5	0.25	2	15	**F-21**	0.901 (0.002)	3.120 (0.002)	**S-21**	0.907 (0.002)	2.873 (0.006)
87.5	12.5	0.75	2	5	**F-22**	0.902 (0.002)	2.870 (0.002)	**S-22**	0.921 (0.002)	2.897 (0.006)
75	25	0.5	1.5	20	**F-23**	0.902 (0.002)	3.020 (0.030)	**S-23**	0.916 (0.005)	3.040 (0.020)
62.5	37.5	0.25	2	15	**F-24**	0.905 (0.002)	3.190 (0.002)	**S-24**	0.922 (0.002)	3.205 (0.002)
62.5	37.5	0.25	2	5	**F-25**	0.914 (0.002)	3.160 (0.009)	**S-25**	0.939 (0.007)	3.197 (0.006)
50	50	0.5	1.5	10	**F-26**	0.913 (0.002)	3.020 (0.002)	**S-26**	0.903 (0.002)	2.980 (0.020)

a: mixture of sugars to reach 50 °Brix in the spreads: 100% of sugar = sucrose or fructose % + isomaltulose %.
Statistical significance *** < 0.001
S: Sucrose; F: Fructose; I: Isomaltulose.
X_1 (% of isomaltulose), X_2 (% of pectin), X_3 (% of citric acid) and X_4 (time of thermal treatment (min)).

Table 3. Retention Index obtained for the volatile compounds identified in the different batches of raw strawberry, concentration of these compounds in raw strawberry expressed as C_F (µg /100 g raw strawberry) and F-ratio obtained from statistical analysis (ANOVA); Changes of volatile strawberry spreads as a consequence of processing expressed as minimum and maximum concentration ratio (C_0-C_F/C_F) (n=3)

Volatile compounds identified in raw strawberry batches				After processing: $\Delta C = \frac{C_0 - C_F}{C_F}$ sucrose-isomaltulose (S)		fructose-isomaltulose (F)	
	RI	**mean**	**F-ratio**	**Minimum**	**Maximum**	**Minimum**	**Maximum**
Esters							
Ethyl acetate	904	129 (61)	4.65**	-0.90	2.85	-0.86	1.95
Isopropyl acetate	911	14 (5)	7.63**	-0.63	2.76	-0.60	2.00
Methyl propionate	926	7 (2)	7.51***	-0.85	62.48	-0.90	2.39
Ethyl propionate	972	2.4 (0.2)	151.06***	-0.15	0.30	-	-
Methyl butyrate	1005	163 (48)	11.8***	-0.48	12.24	-0.53	16.06
Ethyl butyrate	1054	67 (12)	27.12***	-0.87	0.95	-0.76	1.73
Butyl acetate	1093	10 (3)	2.11^{ns}	-0.74	1.84	-0.72	2.01
Methyl hexanoate	1205	23 (6)	3.33*	-0.86	0.87	-0.62	1.40
Butyl butyrate	1217	5 (2)	3.95*	-0.92	0.48	-0.87	0.15
Ethyl hexanoate	1226	24 (4)	32.69**	-	-	-	-
Hexyl acetate	1248	25 (7)	4.48**	-0.86	3.18	-0.72	6.64
3-Hexen-1-yl, acetate	1266	4.1 (0.2)	1716***	-	-	-	-
2-Hexen-1-yl, acetate	1277	48 (12)	6.03**	-0.85	22.09	-0.96	39.62
Hexyl butyrate	1432	5 (2)	5.76**	-0.95	-0.30	-0.88	0.16
Octyl acetate	1494	8 (2)	4.29*	-0.92	-0.25	-0.85	0.74
Butyl octanoate	1634	6.7 (07)	20.59***	-0.88	-0.65	-0.98	-0.43
Alcohols							
Propan-2-ol	943	1.31 (0.07)	30.03***	63.96	311.98	61.15	271.61
Ethanol	953	23 (7)	28.46***	-0.71	11.74	-0.64	14.72

1-Butanol	1169	3.2 (0.4)	68.62***	-0.97	60.49	-0.84	71.88
1-Penten-3-ol	1182	3.3 (0.5)	4.27**	-0.77	27.35	-0.70	24.65
3-Methyl-1-butanol	1210	45 (11)	7.62*	-0.98	-0.80	-1.00	-0.90
1-Pentanol	1236	3.3 (0.4)	37.36***	-0.91	22.12	-0.70	21.54
1-Hexanol	1286	67 (11)	23.46***	-0.96	0.13	-0.93	0.17
3-Hexen-1-yl	1302	3 (1)	1.21 ^{ns}	-	-	-	-
2-Hexen-1-yl	1425	14 (4)	3.54*	-0.81	3.75	-0.80	5.30
Linalool	1564	5 (1)	4.39**	-0.66	1.04	-0.44	1.63
Aldehydes							
Hexanal	1106	29 (6)	7.51***	-0.55	20.05	-0.400	13.33
2-Hexenal	1224	39 (9)	2.2 ^{ns}	-0.46	7.06	0.500	3.09
Ketones							
1-Penten-3-one	1042	4.3 (0.9)	22.54***	-0.93	1.51	-0.90	1.83

Mean (standard deviation).

F-ratio, estimates between groups and within groups ratio (**p-value:** statistical significance with 95 % of confidence, ns: no significance; *< 0.05; **< 0.01; ***<0.001)**.**

C_0: concentration of volatile compound (VC) in the strawberry spread expressed as μg de VC / 100 g of raw strawberry.

C_F: concentration of volatile compound (VC) in the correspondent batch of raw strawberry expressed as μg de VC / 100 g of raw strawberry.

Table 4: Retention Index obtained for the new volatile compounds identified after processing; maximum and minimum concentration values of these compounds within the different formulations expressed as µg of VC / 100 g of spread fruit (n=3).

Volatile compounds after processing (µg of VC / 100 g of fruit spread)					
		sucrose-isomaltulose (S)		**fructose-isomaltulose (F)**	
	RI	**Minimum**	**Maximum**	**Minimum**	**Maximum**
Aldehydes					
2-Methylbutanal	930	1.62	45.00	1.11	34.85
3-Methylbutanal	934	0.00	8.30	1.26	12.15
Octanal	1256	0.00	1.38	0.00	0.96
Nonanal	1418	0.35	2.07	0.35	2.04
Decanal	1524	0.34	3.57	0.30	2.72
Benzaldehyde	1571	0.25	1.34	0.30	1.40
Alcohols					
Eucalyptol	1215	0.00	5.07	0.00	4.79
Furans					
Furfural	1503	9.77	216.05	19.79	297.74
2-Acetilfurane	1539	0.06	8.69	0.29	19.45
5-Methyl furfural	1617	0.11	7.25	0.18	23.03
Mesifurane	1632	0.00	3.97	0.38	3.69
Terpens					
Limonene	1208	0.18	2.57	0.26	6.42
Nitriles					
Acetonitrile	1031	0.00	11.31	0.56	4.40