Spray Drying of Goat Milk Protein Hydrolysates with Angiotensin Converting Enzyme Inhibitory Activity

Short running head: Drying of ACE inhibitory hydrolysates.

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ABSTRACT

A goat milk hydrolysate was stabilized by spray drying using different inlet air temperatures (170-230 ºC) and feed flow rates (4-12 mL/min) following a central composite experimental design. In order to evaluate the effect of operational conditions on process yield, powder properties (density, moisture, hygroscopicity and solubility) as well as angiotensin converting enzyme (ACE) inhibitory activity, experimental data were analyzed by response surface methodology. Input variables showed a significant influence on yield, density and moisture, while hygroscopicity, solubility and ACE inhibitory activity were not affected. The dried hydrolysate presented an average IC_{50} value of 273.13 µg/mL, which involved a loss of 25% in ACE inhibitory activity with respect to the feed. The variations detected in the low molecular weight fractions of the dried hydrolysates could be responsible for the variations in ACE inhibitory activity.

KEYWORDS

Spray-drying; goat milk protein; enzymatic hydrolysis; ACE inhibitory activity; response surface methodology.

INTRODUCTION

Bioactive peptides are defined as peptides that, apart from their nutritional value, originate some physiological benefits (Hartmann and Meisel, 2007). One of the most relevant bioactivities is the demonstrated capacity of reducing blood pressure. Indeed, hypertension is one of the main risk factor for cardiovascular diseases (Mancia et al., 2009).

The potential of these antihypertensive peptides in commonly estimated in vitro by determining their capacity of inhibition on the angiotensin converting enzyme (ACE). This enzyme acts in the renin-angiotensin system transforming angiotensin I into a strong vasoconstrictor denominated angiotensin II (Johnston, 1992). Moreover, ACE degrades bradykinin in the kallikrein–kinin system removing its vasodilator characteristics (Campbell, 2003). Therefore, ACE inhibitory peptides able to reach the blood stream would guide to a reduction in blood pressure.

In their primary structure, some food proteins contain sequences of amino acids that, when released by hydrolysis or fermentation processes, exert a significant antihypertensive activity (Erdmann et al., 2008). Most of published research on ACE inhibitory peptides is focused on their production and identification (Otte et al., 2007; Ruiz-Giménez et al., 2012).

However, only a few studies have considered the stabilization of food protein hydrolysates containing antihypertensive peptides by means of drying (He et al., 2008; Contreras et al., 2011; Amighi et al., 2013). Undoubtedly, an efficient drying procedure is highly desirable since it prolongs the shelf life, reduces transportation costs and improves mixing (Sokhansanj and Jayas, 2006). In this context, spray drying is a good alternative to freeze drying due to lower operation costs subject to producing high

quality products (Gharsallaoui et al., 2007). In the case of antihypertensive peptides, the spray drying process must be able to maintain a major percentage of the ACE inhibitory activity present in the original liquid hydrolysate.

Basically, the spray drying process consists of the pulverization of a liquid feed into a hot air flow. As a result of the small particle size generated in the atomization, the mass transfer rate is considerably high, which results in reduced contact times without significant thermal degradation. Consequently, spray drying represents a convenient method for drying heat-sensitive foods, biologic products, and pharmaceuticals (Filková et al., 2006; Murugesan & Orsat, 2012)

To the best of the authors' knowledge, there is no literature dealing with the modeling of spray drying of milk protein hydrolysates presenting ACE inhibitory activity. Therefore, the aim of this work was to study the effect of some relevant operational variables of the spray drying process on the characteristics of the dried hydrolysate product. As an empirical approach, response surface methodology was used to this purpose.

MATERIALS AND METHODS

Preparation of goat caseins hydrolysate

Goat milk (25.1 g protein/L) was centrifuged (4845 g for 45 min) and then filtrated in the concentration mode through a 0.14 μm ceramic membrane of 1.2 m length, 3 channels and a filtration area of 0.045 m^2 (TAMI, Nyons, France). The filtration was carried out at 50 °C, with a cross-flow of 400 L/h and a transmembrane pressure of 1 bar until reaching a concentration factor equal to 3.

The retentate (mainly constituted by caseins) was hydrolysed by the simultaneous action of subtilisin (Alcalase 2.4 L FG; EC 3.4.21.62) and pancreatic trypsin (PTN 6.0 S Saltfree; EC 3.4.21.4). Both proteases were from Novozymes (Bagsvaerd, Denmark). The hydrolysis was carried out in a tank reactor of 2 L immersed in a thermostatic bath at 50 ºC. Each enzyme was employed at 5 g/L and the pH was maintained at 8 by the controlled addition of 1 N NaOH with an automatic titrator (718 Stat Titrino, Metrohm; Herisau, Switzerland). The reaction was monitored by pH-stat method (Adler-Nissen, 1986). After 3 h of reaction, the enzymes were thermally deactivated.

Spray drying

The spray drying process was performed in a Buchi 190 lab scale spray drier (Flawill, Switzerland). For each experiment, 200 mL of hydrolysate at room temperature (23 ºC) were fed to the equipment. Atomization was produced by a spray nozzle two-fluid atomizer with an orifice of 0.5 mm in diameter employing a compressed air flow rate of 0.5 m^3/h. Air flow was fixed at 25 m^3/h in all the experiments. For each experiment, yield was determined as the percentage of solid recovered as dry product with respect to the initial content of solids in the hydrolysate.

The variables studied were inlet air temperature (T) and feed flow rate (F). Inlet air temperature was assayed in the range 170 – 230 ºC, while feed flow rate was varied from 4 to 12 mL/min. The inlet air temperature range was selected according to the values recommended in the literature for milk spray drying (Bylund, 2003; Brennan, 2006). Performing preliminary tests, undesirable condensations were detected in the drying chamber at 170 ºC and feed flow rates higher than 12 mL/min. On the other hand, excessive browning occurred at 230 ºC for feed flow rates lower than 4 mL/min.

A face-centered central composite design with 3 repetitions of the central point was executed. Although a rotable central composite design would present some desirable statistical properties, the application of the axial factor (α = 1.4) would involve to perform experiments outside of the limits established in the preliminary tests. This could lead to the appearance of condensation and/or excessive browning. Face-centered central composite designs have been successfully employed in both the analysis (e.g. Zhang & Zhang, 2008) and processing (e.g. Oms-Oliu et al., 2009) of food materials.

Analysis of powders

Bulk density: One gram of the powder product was placed in a 10-mL graduated cylinder in order to determine the volume occupied by the dried hydrolysate. The bulk density was determined from the mass–volume ratio.

Moisture: An infrared balance (AD-4714A, Tokyo, Japan) was employed to measure the water content of the hydrolysate. The samples were heated at 105 ºC for 90 min.

Hygroscopicity was determined as described by Cai & Corke (2000). A powder sample was placed into a small dish, weighed and introduced in a desiccator containing a saturated salt solution NaCl, which provided a relative humidity of 75.5% at 20 ºC (Greenspan, 1977). The samples were kept under these conditions until constant weight. Hygroscopicity was expressed as grams moisture per 100 grams of dry solids.

Solubility was determined as described by Amiri-Rigi et al. (2012). Ultrapure water (30 mL) was added to 1 g of hydrolysate and agitated at 500 rpm and 20 ºC in a shaker (Unimax 1010, Heidolph; Schwabach, Germany) for 5 min. Then the mixture was centrifuged at 3000g for 5 min. A 15-mL aliquot of the supernatant was transferred to a

previously weighed Petri dish and dried in an oven at 110 ºC for 4 h. The solubility was expressed as the percentage of total solids recovered in the supernatant.

ACE inhibitory activity

The method proposed by Shalaby et al. (2006) was employed to determine the ACE inhibition capacity of the hydrolysates. The hydrolysis of the substrate (FAPGG, Sigma-Aldrich, St. Louis MO, USA) by ACE (EC 3.4.15.1, Sigma-Aldrich, St. Louis MO, USA) was carried out in a 96-well microplate at 37 ºC by monitoring the absorbance at 340 nm in a Multiskan FC microplate photometer (Thermo Scientific, Finland).

The IC_{50} was determined by representing inhibition percentage versus the logarithm of the protein concentration. The linear regression of the linear zone of this graph allowed to determine the concentration which reduces the enzyme activity by 50%.

Size exclusion chromatography

A water solution of each sample of dried hydrolysate (6.5 g protein/mL) was fractionated by size exclusion chromatography (SEC) using an FPLC system (AKTA purifier UPC 100, GE Healthcare, Uppsala, Sweden) mounted with a Superdex Peptide 10/300 GL column (GE Healthcare, Uppsala, Sweden). Five hundred microliters of the sample solution were injected and eluted with ultrapure water at a flow rate of 0.5 mL/min. The effluent was monitored at 280 nm and the fractions were automatically collected according to the slope changes in the chromatogram. The area of each fraction was integrated using the Unicorn 5.1 software (GE Healthcare, Uppsala, Sweden). The standards employed were ribonuclease A (MW=13700, elution volume=13.82 mL), vitamin B_{12} (MW=1355.37, elution volume=16.64 mL), Gly-Gly-Gly (MW=189.17,

elution volume=18.01 mL) and Gly (MW=75.07, elution volume=18.93 mL), all from Sigma-Aldrich (St. Louis MO, USA).

Statistical analysis

Response surface methodology was employed to determine the effect of the inlet air temperature (T) and feed flow rate (F) on yield (Y), density (D), moisture (M), higroscopicity (H), solubility (S) and ACE inhibitory activity (IC_{50}). It was also studied the variation in the areas the SEC fractions. Input variables were related to output variables by second order polynomials (Eq. 1) using multiple linear regression:

$$Y = b_0 + b_1 \cdot T + b_2 \cdot F + b_{11} \cdot T^2 + b_{12} \cdot T \cdot F + b_{22} \cdot F^2 \qquad (1)$$

Where b_0 is a constant, b_1 and b_2 are the linear coefficients, b_{11} and b_{22} are the quadratic coefficients and b_{12} is the cross-product coefficient.

Analysis of variance (ANOVA) was employed to determine the significance of each term on each output variable. Setting a level of confidence of 95%, those terms having a p-value lower than 0.05 will be statistically significant.

Optimization calculations were performed by using the corresponding second order polynomials. The ranges of the experimental variables assayed were fixed as lower and upper bounds.

RESULTS AND DISCUSSION

The retentate obtained after filtration, with a protein content of 63.5 g/L,) was hydrolyzed reaching a degree of hydrolysis of 35% after 3 h of reaction. According to Adler-Nissen (1986), the average peptide chain length (PCL) of the hydrolysates would

be around 3 amino acid residues. This number represents a favorable peptide size for ACE inhibitory activity since most of the ACE inhibitors present short chains (López-Fandiño et al., 2006). This result is in good agreement with that reported by Cai et al. (2013), who positively related high degrees of hydrolysis with ACE inhibitory activity.

The results obtained after executing the drying experiments are shown in Table 1. With respect to the values outlet air temperature, this variable ranged from 86 to 152 ºC.

Yield

During the drying process, some part of the product remains attached by adhesion to the walls of the drying chamber, causing products loses. As a consequence, yields obtained in the experiments were ranged between 5.9 y 42.5%, which point towards the substantial influence of the operational conditions in the process yield. From an operational point of view, it is crucial to establish a relation between input variables and yield. Therefore, experimental data were adjusted to a polynomial equation obtaining the coefficients shown in Table 2. Two of the five terms included in the second- order equation (inlet air temperature and the interaction term) had a p-value below 0.05, involving significant effect on yield. The model adjusted properly the data, indeed the model obtained was able to explain 88.6% of the experimental variation.

According to the contour plot corresponding to this output variable (Figure 1.a), increasing inlet air temperature seems to be detrimental for yield, which could be related to an increment in the sticky nature of the dried particles. Similar results have been observed previously by others authors (Kurozawa et al., 2011) when chicken protein hydrolysate was assayed. According to Hennings et al. (2001), the temperature of transition from the non-sticky to the sticky state (the sticky-point temperature) is

correlated to the glass transition point. The higher the differences between outlet air temperature and glass transition temperature, the higher the degree of stickiness.

On the other hand, with respect to feed flow rate, yield is generally improved at lower values of this input variable. The smaller size of the particles atomized at low flow improved the efficiency of heat and mass transfer. The fast drying would cause a rapid solidification of the surface which avoids the adhesion of particles on the drier surfaces. Moreover, high feed flow rate combined with low inlet air temperatures is a cause of condensations in the drying chamber, reducing the powder recovery.

The optimum values of the input variables (identified with a mark in Figure 1a) were located at 170 ºC y 4.35 mL/min, which corresponds to a yield of 38.4%. Comparing this yield results to other spray drying processes applied to protein hydrolysates, it can be mentioned the10-15% yield obtained by Abdul-Hamid et al. (2002) for fish hydrolysates or the much higher 74% value reported by Kurozawa et al. (2011), who employed chicken meat hydrolysates.

Density

Experimental values for density ranged between 0.126 and 0.314 g/mL (Table 1). The coefficients of the model which fitted density data are presented in Table 2. The standardized effect determined for temperature had a p-value lower than 0.05, then temperature had a significant effect over the final bulk density of the powder. The model fitted adequately the experimental data, obtaining a coefficient of determination of 0.736.

As it is shown in the contour plot (Figure 1.b), high inlet air temperature reduced the density of the dried product. The same effect of this input variable was reported for by

inlet temperature in bulk density of powders has been obtained for whey protein hydrolysate (Yoo et al., 2009). Although the effect of feed flow rate over bulk density was not significant ($p > 0.05$), a slight positive variation was observed, which could be explained by the generation of particles with a porous structure and low density as a consequence of a fast evaporation (Telang and Thorat, 2010).

The desirable values of density can vary depending on the final use of the product. However, powders with a high density are usually preferred for packaging as well as for reducing the entrainment losses (Yoo et al., 2009). According to the model obtained, the highest density was obtained at the lower inlet air temperature bound (170 ºC) and at the upper feed flow rate bound (12 mL/min), which resulted in a density value of 0.334 g/mL (Figure 1b).

Moisture

The moisture values ranged from 4.3 to 9.1 5 and were similar to those published by Schuck et al. (2007) for caseinate powders or Favaro-Trindade et al. (2010) for casein hydrolysates.

After fitting the experimental data to a quadratic equation (Table 2), four effects (all but the T^2 term) resulted significant. In particular, the linear temperature term presented the lowest p-value, which points toward temperature as the key parameter for the control of the final product moisture. The goodness of the fit was satisfactory since it was able to explain 92.1% of the experimental variation.

Several authors (Tonon et al., 2008; Mestry et al., 2011) found that an increase in inlet air temperature produced a powder with lower moisture content. The same behavior was obtained in this work for goat milk hydrolysates. In the contour plots (Figure 1c), it can

be observed that increasing inlet air temperature would produce a decrease in moisture, reaching a minimum at the highest inlet air temperature assayed. The effect of temperature was much more appreciable at high feed flow rates. In contrast, at lower feed flow rates, the amount of water to be removed would be low and, even employing low inlet air temperatures, the resulting moisture of the final powder would be reduced. With respect to the location of the optimal operational conditions that resulted in minimum moisture, they were 230 ºC and 8.18 mL/min for inlet air temperature and feed flow rate, respectively.

Higroscopicity

According to Kurozawa et al. (2009), protein hydrolysates containing low-molecular-weight peptides present high hygroscopicity. These authors determined a hygroscopicity of 40.9 g water/100 g solids for a meat protein hydrolysate. Using similar analysis conditions, the values obtained in this work for goat milk casein hydrolysates were lower (and consequently, better) since they ranged between 28 and 31 g water/ g solids (Table 1). Such a narrow interval of variation suggested that neither inlet air temperature nor feed flow rate exercised influence on hygroscopicity. Effectively, none of the effects (linear, quadratic or interaction) presented a p-value lower than 0.05. In the same line, the model equation was unsuccessful when trying to fit the experimental data since a coefficient of determination of 0.559 only was calculated. Therefore, it can be said that the hygroscopicty of the final product laid on an average value of 29 g water/ g solids.

Solubility

Spray drying may generate insoluble materials because of protein denaturation which would reduce the powder solubility (Yoo et al., 2009; Mestry et al., 2011). Since hydrolysis process reduced the size of the goat caseins until a PCL of 3 in this work, denaturation would not be a major problem during the drying process. All the solubility values obtained were rather high (87 - 92%), reaching experimental maximum at 170 ºC and 4 mL/min. However, the fitting equation calculated (Table 2) was not acceptable, since the model only was able to explain 45.7% of the experimental variation only. Again, none of the effects was significant at a 95% level. An average value for solubility could be calculated at 90%.

A similar result for the determination coefficient (64.5%) was obtained by Amiri-Rigi et al. (2012) in the spray drying of low-phenylalanine skim milk. On the other hand, these authors found a significant contribution for both linear terms of inlet air temperature and feed flow rate.

ACE inhibitory activity

The ACE inhibitory activity of the hydrolysates could be reduced to some extent due to the high temperatures reached in the drying chamber. In order to determine the effect of the operational conditions (inlet air temperature and feed flow rate) on the ACE inhibitory activity of the hydrolysates, the variations on the IC_{50} values were also fitted to a quadratic equation. The percentage of variation on the IC_{50} was calculated according to Eq. 2.

$$\Delta IC_{50} = \frac{(IC_{50} - IC_{50i})}{IC_{50i}} \times 100 \qquad (2)$$

where IC_{50i} is the IC_{50} value of the hydrolysate before spray drying (218.50 µg/mL) and IC_{50} is the ACE inhibitory activity of each dried hydrolysate obtained in powder form.

In this case, the goodness of the fit was very low (Table 2) since the model could only explain 27.9% of the experimental data. Moreover, the p-value determined for each effect was higher than 0.05 which means that none of them had a significant effect over ΔIC_{50}. It can be concluded that, in our study, neither T nor F have a significant effect over the ACE inhibitory activity of dried hydrolysates. Therefore, there were not statistical differences between the ΔIC_{50} of the experiments. Taking into account this, the variations on ACE inhibitory activity of the dried hydrolysates could be estimated as the average of ΔIC_{50} of all the experiments, which resulted in an increase of 25% in the IC_{50} values of the dried hydrolysates.

When comparing to published literature dealing with the spray drying of antihypertensive hydrolysates, He et al. (2008) did not found any reduction in ACE inhibition capacity for shrimp hydrolysates, despite of the slight variations observed in the peptide profiles. Contreras et al. (2011) studied casein hydrolysates concluding that the two relevant peptides resisted the spray drying without loss of inhibition capacity. Amighi et al. (2013) studied the spray drying of ACE inhibitory enzyme-modified white cheese. As in this work, their results showed that bioactivity decreased significantly due to spray drying. A justification was given due to the formation of aggregates which could not participate in inhibition in vitro. However, after gastric digestion, the peptides could be again accessible for in vivo activity.

Size exclusion chromatography

With the objective of studying the possible changes produced in the peptides, each dried hydrolysate was subject to size exclusion chromatography. All the chromatograms obtained for the dried hydrolysates presented a similar shape when compared to the chromatogram of the original (not spray-dried) hydrolysate (see the examples provided

in Figure 2). The percentages of the total area of the 8 fractions (A-H) delimited in Figure 2 were determined for each experiment and the percentages of variation in area (ΔA) were calculated by Eq. 3:

$$\Delta A = \frac{(A - A_i)}{A_i} \times 100 \qquad (3)$$

where A and A_i are the area percentages of the hydrolysate fraction after and before spray drying, respectively.

As well as ACE inhibitory activity, the ΔA values calculated for each fraction (Table 3) were correlated to the operational conditions (inlet air temperature and feed flow rate) by a quadratic model. In Table 2, it is shown the coefficient obtained and the p-value for each effect determined with the ANOVA analysis. Only fractions C (58-5.7 kDa), E (2-0.9 kDa) and H (< 90 Da) were properly adjusted to the input variables (inlet air temperature and feed flow rate). For these variables, as it can be observed in Table 4, the p-values of at least one term was lower than 0.05. Also, their coefficients of determination reached acceptable values: 84.2%, 90.3% and 79.8% for C, E and H fractions, respectively. On the other hand, the variations in area of fractions A, B, D, F and G were not influenced by input variables.

In the contour plots shown in Figure 3, it can be observed that temperatures higher than 190 ºC usually increased the area of fractions C and E. With respect to fraction H, a pronounced increase in the area was always observed, but higher temperatures generated smaller increases. The maximum area increase for C and E were observed at 230 ºC, while for fraction H was determined at 170 ºC. Nevertheless, the changes produced in these fractions (C, E and H) would not influence directly the ACE inhibitory activity since the IC_{50} was not affected by the input variables. Therefore, the reduction in ACE inhibition detected in spray dried hydrolysates could be originated by

the changes produced in the other fractions. Especially due to the reduction in the area produced in fractions F (0.9 – 0.3 kDa) and G (300 – 90 Da). These fractions present small peptides which a strong ACE inhibitory activity (Espejo-Carpio et al., 2013). Consequently, a decrease in the fraction area could be related to a reduction in ACE inhibitory which would be independent of both inlet air temperature and feed flow rate.

CONCLUSIONS

A goat milk casein hydrolysate (DH = 35%) was successfully stabilized by spray drying at different conditions of inlet air temperature and feed flow rate. Yield, bulk density and moisture were adequately fitted to a quadratic equation which allowed their optimization. The maximum yield (38.4 %) was reached at inlet air temperature of 170 ºC and at feed flow rate of 4.35 mL/min. According to the models obtained, the bulk density and moisture would have a value of 0.261 g/mL and 5.6 %, respectively. Other measured variables, such as hygroscopicity and solubility presented average values of 29 g water/ g solids and 90 %, respectively. ACE inhibitory activity was not fitted satisfactorily, since inlet air temperature and feed flow rate did not affect the ACE inhibitory activity of the dried hydrolysates, which experienced an average decrease of 25% in its IC_{50} value. According to the size exclusion chromatograms, alteration in low molecular weight peptides (lower than 900 Da) of the original hydrolysate could be responsible for the loss in ACE inhibitory activity.

REFERENCES

Abdul-Hamid A, Bakar J, & Bee GH (2002) Nutritional quality of spray dried protein hydrolysate from Black Tilapia (Oreochromis mossambicus). Food Chemistry, 78(1), 69-74.

Adler-Nissen J (1986) Enzymic Hydrolysis of Food Proteins. Elsevier Science, London UK.

Amighi F, Emam-Djomeh Z, & Madadlou A (2013) Spray drying of ACE inhibitory enzyme-modified white cheese. International Journal of Food Science & Technology, (In press).

Amiri-Rigi A, Emam-Djomeh Z, Mohammadifar MA, & Mohammadi M (2012) Spray drying of low-phenylalanine skim milk: optimisation of process conditions for improving solubility and particle size. International Journal of Food Science & Technology, 47(3), 495–503.

Brennan JG (2006) Food Processing Handbook, pp 117-118. Wiley-VCH, Weinheim, Germany.

Bylund MS (2003) Dairy Processing Handbook, pp 261-373. Tetra Pak Processing Systems, Lund, Sweden.

Cai YZ & Corke H (2000) Production and properties of spray-dried Amaranthus betacyanin pigments. Journal of Food Science, 65(7), 1248-1252.

Cai L, Xiao L, Liu C & Ying T (2013) Functional Properties and Bioactivities of Pine Nut (Pinus gerardiana) Protein Isolates and Its Enzymatic Hydrolysates. Food and Bioprocess Technology, 6 (8), 2109-2117.

Campbell DJ (2003) The renin-angiotensin and the kallikrein-kinin systems. The International Journal of Biochemistry & Cell Biology, 35(6), 784-791.

Contreras M del M, Sevilla MA, Monroy-Ruiz J, Amigo L, Gómez-Sala B, Molina E, Ramos M & Recio, I (2011) Food-grade production of an antihypertensive casein hydrolysate and resistance of active peptides to drying and storage. International Dairy Journal, 21(7), 470-476.

Erdmann K, Cheung BWY & Schröder H (2008) The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. The Journal of Nutritional Biochemistry, 19(10), 643-654.

Espejo-Carpio FJ, De Gobba C, Guadix A, Guadix EM, & Otte J (2013) Angiotensin I-converting enzyme inhibitory activity of enzymatic hydrolysates of goat milk protein fractions. International Dairy Journal, 32(2), 175-183.

Favaro-Trindade CS, Santana AS, Monterrey-Quintero ES, Trindade MA & Netto FM (2010) The use of spray drying technology to reduce bitter taste of casein hydrolysate. Food Hydrocolloids, 24(4), 336-340.

Filková I, Huang LX & Mujumdar AS (2006) Industrial Spray Drying Systems. In Mujumdar (ed) Handbook of Industrial Drying, pp. 215-256. CRC Press, Florida, USA.

Gharsallaoui A, Roudaut G, Chambin O, Voilley A & Saurel R (2007) Applications of spray-drying in microencapsulation of food ingredients: An overview. Food Research International, 40(9), 1107-1121.

Greenspan L (1977) Humidity fixed points of binary saturated aqueous solutions. Journal of Research of the National Bureau of Standards, 81 A(1), 89-96.

Hartmann R & Meisel H (2007) Food-derived peptides with biological activity: from research to food applications. Current Opinion in Biotechnology, 18(2), 163-169.

He H-L, Wu H, Chen X-L, Shi M, Zhang X-Y, Sun C-Y, Zhang Y-Z & Zhou B-C (2008) Pilot and plant scaled production of ACE inhibitory hydrolysates from Acetes chinensis and its in vivo antihypertensive effect. Bioresource Technology, 99(13), 5956-5959.

Hennigs C, Kockel TK & Langrish TAG (2001) New measurements of the sticky behavior of skim milk powder. Drying Technology, 19(3-4), 471-484.

Johnston CI (1992) Renin-angiotensin system: A dual tissue and hormonal system for cardiovascular control. Journal of Hypertension, 10(7), S13-S26.

Kurozawa LE, Park KJ & Hubinger MD (2009) Effect of carrier agents on the physicochemical properties of a spray dried chicken meat protein hydrolysate. Journal of Food Engineering, 94(3–4), 326-333.

Kurozawa LE, Park KJ & Hubinger MD (2011) Spray Drying of Chicken Meat Protein Hydrolysate: Influence of Process Conditions on Powder Property and Dryer Performance. Drying Technology, 29(2), 163-173.

López-Fandiño R, Otte J & van Camp J (2006) Physiological, chemical and technological aspects of milk-protein-derived peptides with antihypertensive and ACE inhibitory activity. International Dairy Journal, 16(11), 1277-1293.

Mancia G, Laurent S, Agabiti-Rosei E, Ambrosioni E, Burnier M, Caulfield MJ, … & Zanchetti A (2009) Reappraisal of European guidelines on hypertension management: a European Society of Hypertension Task Force document. Journal of Hypertension, 27(11), 2121–2158.

Mestry AP, Mujumdar AS & Thorat BN (2011) Optimization of Spray Drying of an Innovative Functional Food: Fermented Mixed Juice of Carrot and Watermelon. Drying Technology, 29(10), 1121-1131.

Murugesan R, Orsat V (2012) Spray Drying for the Production of Nutraceutical Ingredients-A Review (Review). Food and Bioprocess Technology, 5(1), 3-14.

Oms-Oliu G, Odriozola-Serrano I, Soliva-Fortuny R & Martín-Belloso O (2009) Effects of high-intensity pulsed electric field processing conditions on lycopene, vitamin C and antioxidant capacity of watermelon juice. Food Chemistry, 115, 1312–1319.

Otte J, Shalaby SMA, Zakora M & Nielsen MS (2007) Fractionation and identification of ACE inhibitory peptides from [alpha]-lactalbumin and [beta]-casein produced by thermolysin-catalysed hydrolysis. International Dairy Journal, 17(12), 1460-1472.

Ruiz-Giménez P, Salom JB, Marcos JF, Vallés S, Martínez-Maqueda D, Recio I, Torregrosa G, Alborch E & Manzanares, P (2012) Antihypertensive effect of a bovine lactoferrin pepsin hydrolysate: Identification of novel active peptides. Food Chemistry, 131(1), 266-273.

Schuck P, Mejean S, Dolivet A, Jeantet R & Bhandari B (2007) Keeping quality of dairy ingredients. Le Lait, 87(4-5), 481-488.

Shalaby SM, Zakora M & Otte J (2006) Performance of Two Commonly Used Angiotensin-Converting Enzyme Inhibition Assays Using FA-PGG and HHL as Substrates. Journal of Dairy Research, 73(02), 178-186.

Sokhansanj S & Jayas DS (2006) Drying of foodstuff. . In Mujumdar (ed) Handbook of Industrial Drying, pp. 521-546. CRC Press, Florida, USA.

Telang AM & Thorat BN (2010) Optimization of Process Parameters for Spray Drying of Fermented Soy Milk. Drying Technology, 28(12), 1445-1456.

Tonon RV, Brabet C & Hubinger MD (2008) Influence of process conditions on the physicochemical properties of açai (Euterpe oleraceae Mart.) powder produced by spray drying. Journal of Food Engineering, 88(3), 411-418.

Yoo SH, Jayaprakasha HM, Lee SY, Paik HD & Yoon YC (2009) Effects of processing parameters of spray drying on some of the quality attributes of whey protein concentrate hydrolysates. Milchwissenschaft, 64(1), 78-81.

Zhang Y & Zhang J (2008) Optimization of headspace solid-phase microextraction for analysis of ethyl carbamate in alcoholic beverages using a face-centered cube central composite design. Analytica Chimica Acta, 627, 212–218.

LIST OF FIGURES AND TABLES

- Figure 1. Contour plots for yield (a), density (b) and moisture (c).
- Figure 2. Size exclusion chromatogram of the original goat casein hydrolysate (a) and the hydrolysates dried at 8 mL/min and 170 ºC (b), 200ºC (c) and 230 ºC (d).
- Figure 3. Contour plots for the relative area increment for the SEC fractions C (a), E (b) and H (c).
- Table 1. Experimental values and standard deviations from triplicate measurements for yield and powder properties with respect to the input variables (inlet air temperature and feed flow rate)
- Table 2. Regression coefficients and (p-values) of the models corresponding to the product properties.
- Table 3. Experimental values and standard deviations from triplicate measurements for the variation of SEC-fractions areas with respect to the input variables (inlet air temperature and feed flow rate)
- Table 4. Regression coefficients and (p-values) of the models corresponding to the variation in chromatographic areas.

Figure 1. Contour plots for yield (a), density (b) and moisture (c).

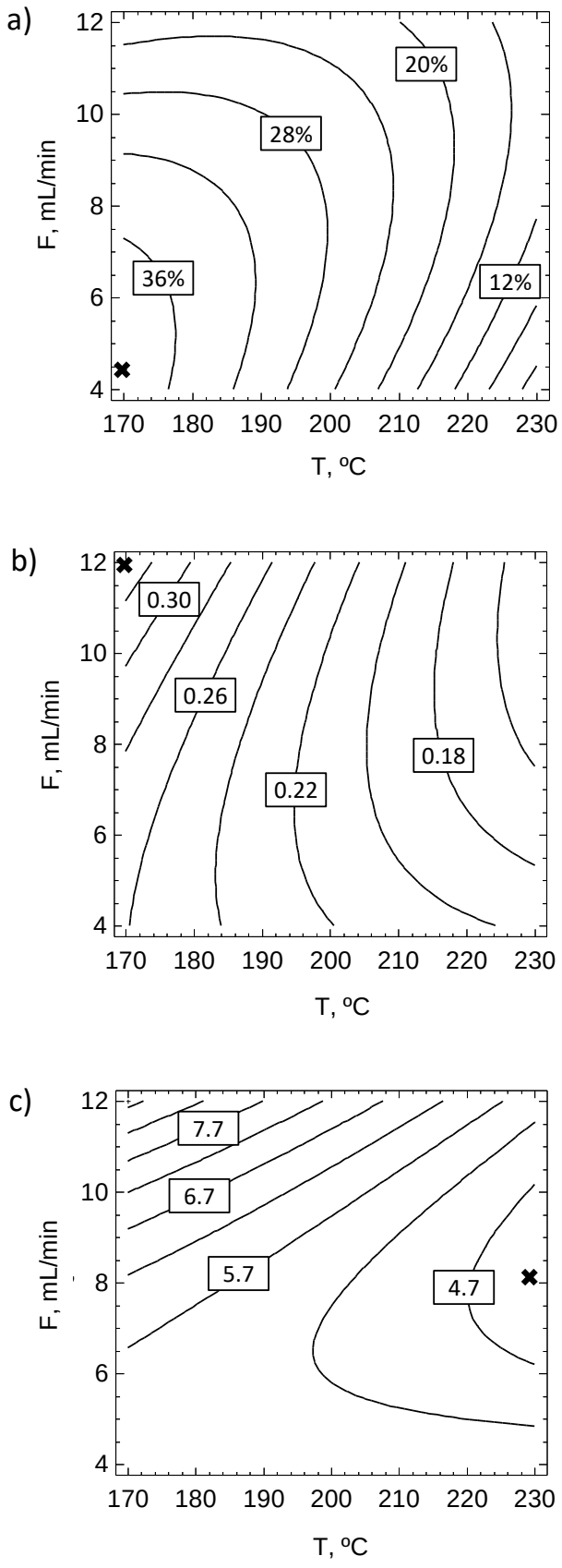

Figure 2. Size exclusion chromatogram of the original goat casein hydrolysate (a) and the hydrolysates dried at 8 ml/min and 170 ºC (b), 200ºC (c) and 230 ºC (d).

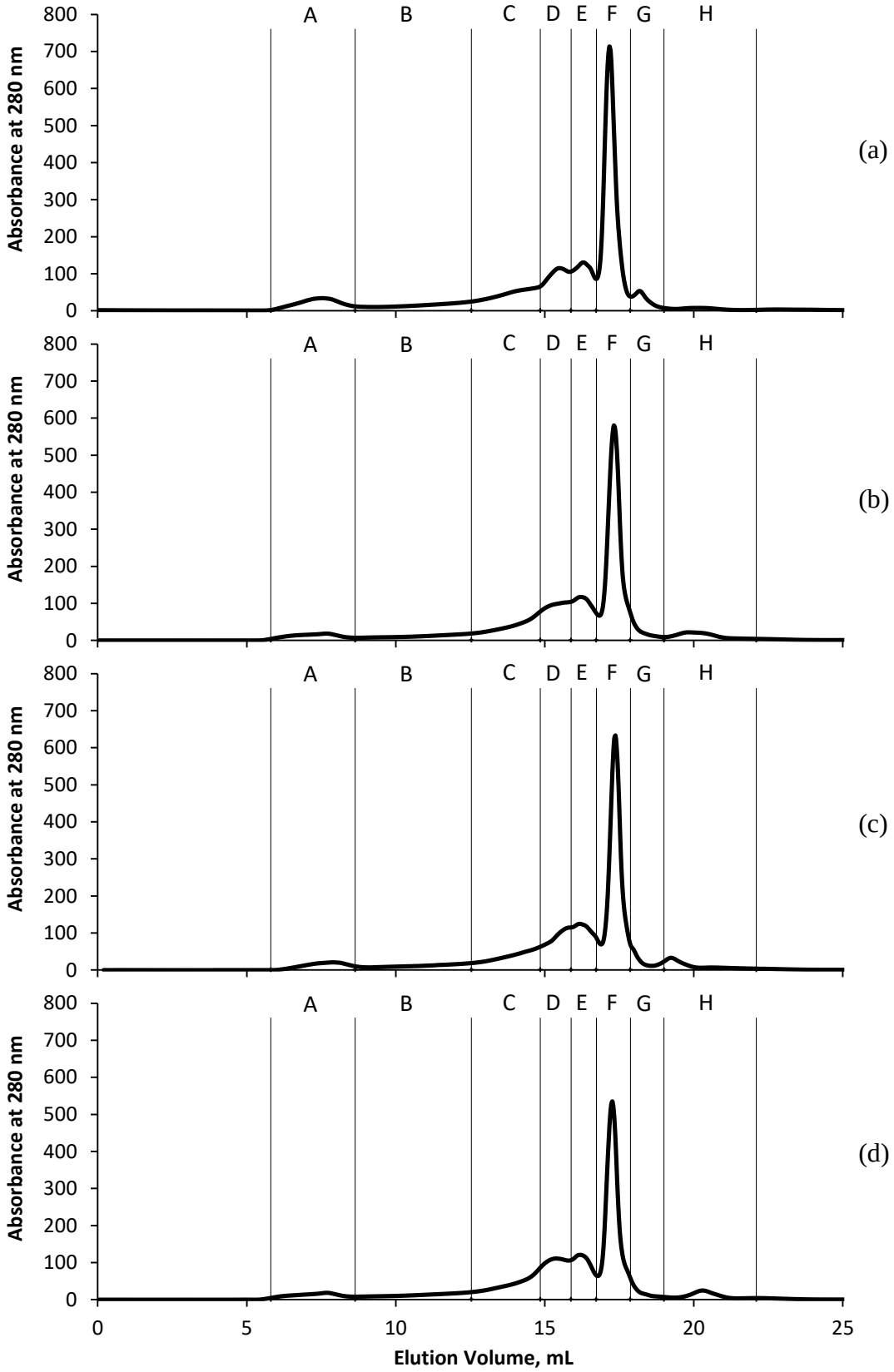

Figure 3. Contour plots for the relative area increment for the SEC fractions C (a), E (b) and H (c).

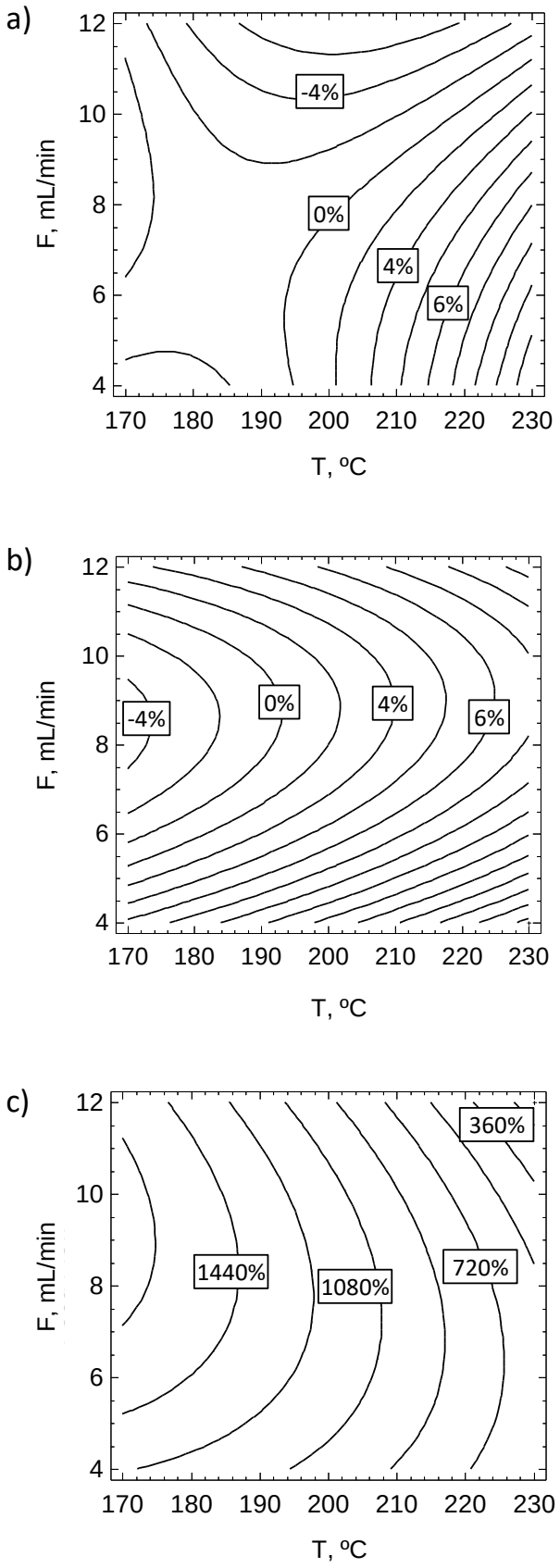

Table 1. Experimental values and standard deviations from triplicate measurements for yield and powder properties with respect to the input variables (inlet air temperature and feed flow rate)

T, ºC	F, L/min	Yield, %	Density, g/mL	Moisture, %	Higroscopicity, g water/ g solids	Solubility, %	ΔIC50, %
170	4	42.50 ± 2.08	0.295 ± 0.009	5.9 ± 0.2	28.672 ± 0.824	92.89 ± 2.10	20.92 ± 0.91
170	8	32.33 ± 1.18	0.267 ± 0.012	5.6 ± 0.2	28.043 ± 0.633	87.33 ± 2.05	36.44 ± 1.66
170	12	20.05 ± 0.78	0.314 ± 0.011	9.1 ± 0.3	28.066 ± 1.073	90.58 ± 4.11	13.06 ± 0.50
200	4	16.56 ± 0.79	0.156 ± 0.006	5.3 ± 0.1	30.510 ± 0.885	91.31 ± 2.82	36.85 ± 0.80
200	8	30.58 ± 1.22	0.241 ± 0.007	5.7 ± 0.1	29.181 ± 0.836	89.46 ± 3.60	10.92 ± 0.34
200	8	29.37 ± 1.20	0.210 ± 0.008	5.0 ± 0.2	29.330 ± 0.960	91.48 ± 4.40	15.74 ± 0.66
200	8	27.01 ± 1.26	0.205 ± 0.007	5.8 ± 0.2	28.640 ± 1.331	91.29 ± 3.35	30.90 ± 0.95
200	12	26.14 ± 0.56	0.274 ± 0.009	6.8 ± 0.2	28.282 ± 1.033	89.55 ± 4.25	19.03 ± 0.89
230	4	5.88 ± 0.28	0.228 ± 0.010	5.7 ± 0.3	29.056 ± 1.069	89.68 ± 2.19	23.78 ± 0.50
230	8	11.14 ± 0.34	0.148 ± 0.006	4.3 ± 0.1	30.724 ± 0.731	88.99 ± 2.43	28.56 ± 1.08
230	12	11.12 ± 0.50	0.126 ± 0.004	5.5 ± 0.2	29.025 ± 1.211	89.52 ± 3.18	39.31 ± 1.07

Table 2. Regression coefficients and (p-values) of the models corresponding to the product properties.

	Yield	**Density**	**Moisture**	**Higroscopicity**	**Solubility**	**ΔIC_{50}**
Constant	-8.25E+00	6.42E-01	2.64E+00	1.78E+01	5.75E+01	2.74E+02
T	1.03E+00	3.74E-03	2.72E-02	1.04E-01	4.38E-01	-2.22E+00
	(0.0036)	(0.0194)	(0.0080)	(0.1063)	(0.5276)	(0.5038)
F	-7.27E+00	3.60E-02	5.02E-01	-1.65E-01	-2.24E+00	-9.98E+00
	(0.5804)	(0.7503)	(0.0132)	(0.2204)	(0.323)	(0.7447)
T^2	4.67E-03	9.00E-06	3.00E-06	-2.28E-04	-1.22E-03	4.86E-03
	(0.2604)	(0.7815)	(0.9935)	(0.7113)	(0.3177)	(0.5878)
F·T	5.77E-02	2.52E-04	-7.08E-03	1.20E-03	4.47E-03	4.87E-02
	(0.0466)	(0.2369)	(0.0177)	(0.7449)	(0.5261)	(0.3759)
F^2	-2.87E-01	9.94E-04	6.89E-02	-1.21E-02	7.33E-02	-1.18E-02
	(0.2242)	(0.598)	(0.0157)	(0.7273)	(0.2892)	(0.9811)
R^2	0.886	0.736	0.921	0.559	0.457	0.279

Table 3. Experimental values and standard deviations from triplicate measurements for the variation of SEC-fractions areas with respect to the input variables (inlet air temperature and feed flow rate)

T, °C	F, mL/min	ΔA_A, %	ΔA_B, %	ΔA_C, %	ΔA_D, %	ΔA_E, %	ΔA_F, %	ΔA_G %
170	4	-44.77 ± 3.52	59.60 ± 5.60	-4.65 ± 0.45	-11.98 ± 1.15	6.22 ± 0.32	-9.32 ± 0.86	19.80 ±
170	8	-43.36 ± 2.18	-2.89 ± 0.15	4.16 ± 0.42	11 ± 0.97	-0.61 ± 0.06	-5.22 ± 0.46	-30.32 ±
170	12	-33.71 ± 2.33	50.66 ± 2.60	-2.21 ± 0.20	0.41 ± 0.03	1.74 ± 0.16	-14.34 ± 1.01	18.63 ±
200	4	-38.34 ± 3.32	-21.83 ± 1.42	0.46 ± 0.04	24.05 ± 1.78	16.8 ± 1.52	-3.54 ± 0.19	-12.12 ±
200	8	-23.32 ± 1.32	9.83 ± 0.87	-1.02 ± 0.06	7.56 ± 0.47	6.48 ± 0.51	-5.27 ± 0.46	-41.85 ±
200	8	-20.00 ± 1.37	49.42 ± 2.78	0.08 ± 0.01	-0.17 ± 0.01	0.02 ± 0.01	-11.38 ± 0.92	-9.93 ±
200	8	-18.90 ± 1.53	55.93 ± 4.63	2.72 ± 0.14	-9.19 ± 0.75	0.01 ± 0.01	-13.31 ± 1.11	11.56 ±
200	12	-43.89 ± 2.66	-10.76 ± 1.06	-9.34 ± 0.59	10.47 ± 0.94	7.58 ± 0.42	-3.84 ± 0.34	13.12 ±
230	4	-54.30 ± 4.56	69.44 ± 4.09	20.55 ± 1.86	31.25 ± 3.06	28.64 ± 2.64	-24.88 ± 2.26	-11.28 ±
230	8	-27.59 ± 2.06	11.52 ± 0.90	3.75 ± 0.37	26.24 ± 1.83	5.81 ± 0.32	-9.62 ± 0.60	-39.00 ±
230	12	19.51 ± 1.07	15.15 ± 1.06	0.22 ± 0.01	14.37 ± 1.25	17.3 ± 1.20	-4.75 ± 0.28	-97.80 ±

Table 4. Regression coefficients and (p-values) of the models corresponding to the variation in chromatographic areas.

	ΔA_A	ΔA_B	ΔA_C	ΔA_D	ΔA_E	ΔA_F	ΔA_G	ΔA_H
Constant	5.57E+01	6.89E+02	1.48E+02	4.58E+01	1.92E+01	-8.88E+01	-9.14E+02	-3.16E+03
T	-6.07E-01	-7.34E+00	-2.03E+00	-1.11E+00	-3.11E-02	1.24E+00	9.28E+00	4.04E+01
	(0.188)	(0.9146)	(0.0457)	(0.059)	(0.0065)	(0.4755)	(0.0857)	(0.01)
F	-1.70E+01	1.93E+01	1.10E+01	8.30E+00	-8.66E+00	-9.74E+00	1.74E+01	5.51E+02
	(0.0978)	(0.6235)	(0.0432)	(0.5699)	(0.0524)	(0.3205)	(0.432)	(0.7613)
T^2	-2.70E-04	2.01E-02	6.40E-03	4.99E-03	9.80E-04	-4.28E-03	-2.18E-02	-1.15E-01
	(0.9815)	(0.5117)	(0.0807)	(0.581)	(0.7423)	(0.3143)	(0.3431)	(0.6398)
F·T	1.31E-01	-9.45E-02	-4.74E-02	-6.10E-02	-1.43E-02	5.24E-02	-1.78E-01	-1.57E+00
	(0.106)	(0.6018)	(0.0422)	(0.2819)	(0.4345)	(0.0704)	(0.2121)	(0.3065)
F^2	-3.68E-01	-1.59E-01	-1.65E-01	1.96E-01	6.54E-01	-7.65E-03	9.71E-01	-1.54E+01
	(0.5819)	(0.9249)	(0.3632)	(0.6978)	(0.0091)	(0.973)	(0.4451)	(0.2888)
R^2	0.682	0.181	0.842	0.628	0.903	0.628	0.637	0.798