PRODUCTION OF A LOW-CHOLESTEROL SHRIMP USING SUPERCRITICAL EXTRACTION

I. HIGUERA-CIAPARA[1], A.R. TOLEDO-GUILLEN, L. NORIEGA-OROZCO, K.G. MARTINEZ-ROBINSON and M.C. ESQUEDA-VALLE

Centro de Investigación en Alimentación y Desarrollo
A.C., Carretera a Varadero Nacional
Km. 6.6, A.P. 284
Guaymas, Sonora, 85480
Mexico

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ABSTRACT

A low-cholesterol shrimp was produced using supercritical extraction. The processing sequence included freeze drying, cholesterol extraction and rehydration. The shrimp was freeze dried, kept under vacuum until an experimental central composite rotatory design was applied using Response Surface Analysis for the supercritical extraction process. Three variables at five levels each were tested during the experiment (pressure, volume and temperature). After the extraction procedure, various rehydration and cooking conditions were applied to obtain a processed product with characteristics similar to those of the natural shrimp. Two sensory analyses were performed: one which compared the attributes of fresh shrimp with those of the freeze-dried and rehydrated products, and another one which compared the acceptability between fresh shrimp and low-cholesterol shrimp after freeze drying, supercritical extraction and rehydration. Under the conditions of 310 bar, 1875 L of carbon dioxide and 37C, it was possible to obtain a low-cholesterol shrimp with acceptable organoleptic properties.

INTRODUCTION

Shrimp has become a commodity in the world market. To a large extent, this is due to the dramatic increases in aquaculture production, particularly in

[1] Corresponding author. TEL: (52)(622)2216533; FAX: (52)(622)2216533; EMAIL: higuera@cascabel.ciad.mx

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developing countries such as Ecuador, Thailand, Indonesia, Mexico and others (Feidi 2003). The U.S. market alone consumes almost 850 million pounds of shrimp per year (National Fisheries Institute 2004), and while prices have marked fluctuations throughout the year, shrimp consumption has continued to expand steadily from year to year.

Shrimp is a low-fat food containing less than 2% of total lipids in the edible portion. However, it is considered a high-cholesterol product, because it contains more than 150 mg cholesterol/100 g of edible portion. Several reports in the scientific literature have discussed the role of dietary cholesterol as a significant risk factor in coronary heart disease (Gylling and Miettinen 2001; Rubio 2002), a major cause of death in the U.S.A. and in Japan (Komatsu and Sakurai 1996; Williams and Bray 2001; Koeller and Talbert 2002). This evidence has led to the development of many low-cholesterol and cholesterol-free foods (Kodali 2001).

In spite of the increasing market demand for shrimp, no reports have been published thus far on processing shrimp with the aim of reducing its cholesterol content. This situation led the authors to work in the process herein described which has been filed for an international patent under the Patent Cooperation Treaty system (international protection of industrial property) (Higuera-Ciapara *et al.* 2002). Supercritical extraction has been used to lower cholesterol in ground beef (Chao *et al.* 1991; King *et al.* 1993; Chao 1996), krill (Yamaguchi *et al.* 1986) and other foods. This technology has had a significant impact at the industrial level for a number of applications, i.e., to produce caffeine-free coffee, to extract pigments and others of similar nature (King 2000). Supercritical extraction consists of heating a fluid above its critical temperature and compressing it above its critical pressure so that the distinction between the liquid and the gas phase disappears, and the fluid can no longer be made liquid by increasing its pressure, neither into a gas by increasing its temperature (Sihvonen *et al.* 1999). Under these conditions, the fluid acquires thermodynamic and transport properties such that its solute mass transfer ratio is considerably larger than in a typical liquid solvent (Rizvi *et al.* 1986). By manipulating the operating conditions during a supercritical extraction process, the supercritical fluid can effectively and selectively extract specific components such as fats, oils, cholesterol, ketones, aldehydes and esters while leaving protein and carbohydrates in their intact state (Dziezak 1986). Because of its nontoxicity, low cost and ease of recovery, the supercritical solvent most widely used in the food industry is carbon dioxide (CO_2).

The objective of this study was to use CO_2 supercritical extraction to obtain a low-cholesterol shrimp while maintaining its sensory properties without significant changes as compared to the fresh product.

MATERIALS AND METHODS

Raw Material Conditioning

Five-pound blocks of frozen shrimp (*Litopenaeus vannamei* and *Litopenaeus stylirostris*) were obtained directly from processing plants in Guaymas, Sonora, Mexico during the months of January through April for the experimental work. The shrimp blocks were kept frozen at −20C until their use.

For all the experimental treatments, shrimp were thawed out at refrigeration temperatures, peeled and packaged in plastic bags to individually freeze them at −40C during a 4-h period in an Ultraquick freezer (Legacy Ultra-Low Technology of GS Laboratory Equipment, Asheville, NC). For an experimental run, shrimp were freeze dried until their moisture content was reduced to 1–5%. This was performed using a laboratory-scale freeze drier equipped with trays (Model VirTis S.P. Industrics, Inc., Warminster, PA). The freeze-dried shrimp were packaged under vacuum using a Digimat Super Vac packaging machine Model GK-185 (Mid Atlantic Equipment Co., Sandston, VA).

Supercritical Extraction

A laboratory-scale dense gas management system (Marc Sims SFE, Berkeley, CA) was used to extract cholesterol from shrimp muscle. The extraction system consisted of four basic components: a compressor or solvent pump, a tubular extractor, a pressure/temperature control system and a glass separator (Fig. 1). CO_2 (99.99% pure) was obtained locally and used as the supercritical fluid for all the experimental runs.

Eight freeze-dried shrimp size 16–20/lb were placed in a 300-mL extraction vessel. The number of shrimp used was determined by the capacity and shape of the extraction vessel. The empty spaces were filled with glass beads. CO_2 was passed through the compressor until the experimental supercritical pressure was achieved. A back pressure regulator was used to maintain the desired supercritical pressure. The gas went into the heated jacketed vessel until the desired operating temperature that was previously established was reached. The measurement of the CO_2 volume used for each experimental run started when the desired pressure and temperature were attained. An expansion valve served as the flow regulator. CO_2 flow through the system was maintained at 5.5–6.2 L/min. The discharge gas and the extract passed through the needle expansion valve toward a U-tube used as a separator. This valve allowed the separation of the fluid by precipitation, thus releasing the extract, i.e., a pressure variation under supercritical conditions implies a reduction in density and thus, a reduction in the solvating capacity. Once this step was finished, the extracted shrimp were packaged under vacuum.

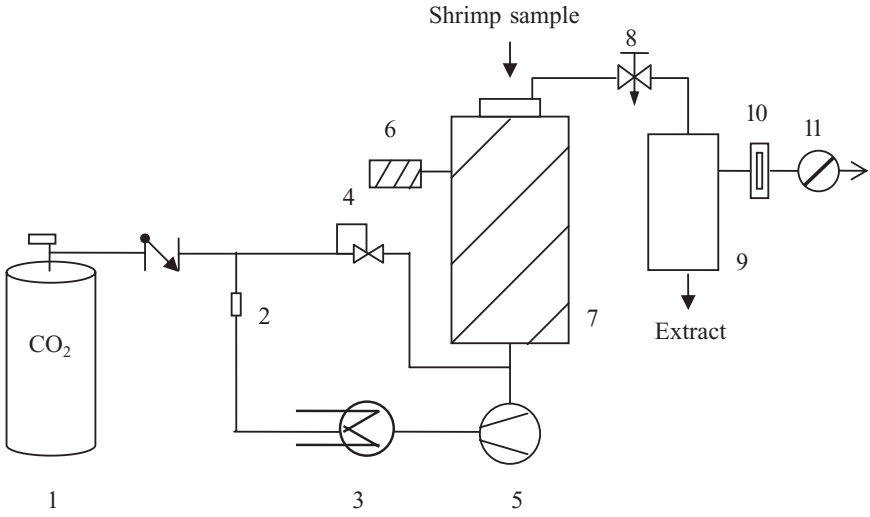

FIG. 1. FLOW DIAGRAM OF THE SUPERCRITICAL EXTRACTION SYSTEM
Gas supply, 1; filter, 2; cooler, 3; pressure regulator, 4; CO_2 pump, 5; heater control, 6; extractor vessel, 7; expansion valve, 8; glass separator, 9; flow meter, 10; flow totalizer, 11.

Once the CO_2 was separated from the extract, it flowed through a measurement device which determined the total volume used for the experiment. The reading corresponded to the CO_2 volume measured at atmospheric pressure and at a temperature of 25C. Such conditions were attained by the fluid after passing through the expansion valve.

Experimental Design

Response Surface Analysis methodology was used to find the optimum operating conditions for cholesterol removal using supercritical extraction. A central composite rotatory design for three independent variables with five levels each was used. The number of experimental points in the design was sufficient to provide a statistical significance ($>0.95\%$) of the quadratic model (Arteaga *et al.* 1994). The variables studied were pressure (275, 289, 310, 331 and 345 bar), CO_2 volume (250, 909, 1875, 2841 and 3500 L) and temperature (35, 36, 37, 38 and 39C). The minimum and maximum levels of the variables were obtained in preliminary trials. The CO_2 volume variable represented the total quantity of the gas used in the experimental run measured at 25C and at atmospheric pressure conditions. The response variable (Y) was the residual cholesterol in shrimp after extraction (on a dry weight basis) and quantified using gas chromatography (GC).

Cholesterol-Content Determination

Extraction Procedure. The method described by Al-Hasani *et al.* (1993) was followed. The same procedure was followed to determine the cholesterol content in the samples of fresh shrimp.

Conditions for Analysis. A gas chromatograph (Varian 3400 CX, Varian Chromatography Systems, Walnut Creek, CA) equipped with a flame ionization detector was used. A capillary column (SAC-5, Supelco, Bellofonte, PA) measuring 30 m in length, 0.25 mm in internal diameter and 0.25 μm in width was used for separation. The column temperature was 300C. Helium flowing at a speed of 1 mL/s was used as carrier gas. The volume of extract injected into the column was 1 μL. The quantitation of cholesterol was performed using the internal standard method.

Shrimp Rehydration

The sample of shrimp that was previously freeze dried and extracted was rehydrated at room temperature using a ratio of 5-mL distilled water/g of shrimp. Rehydration was carried out under a vacuum (21″ Hg). This was achieved by placing the shrimp in a container and applying vacuum for 1 h. The shrimp were then turned on its side and rehydrated under the same conditions for an additional 1 h. The rehydrated and cooked shrimp were packaged in a Cryovac plastic bag and frozen individually at –40C.

Sensory Analysis

Two sensory analysis tests were applied. In the first, a duo–trio test was used to assess the organoleptic attributes of the freeze-dried rehydrated product in comparison to the fresh shrimp. A 30-member untrained panel was used for this purpose. The second type of sensory analysis that was used was an acceptance sensory test. This was applied to the product which had been previously freeze dried, supercritically extracted and rehydrated. Again, a 30-member untrained panel was used.

Statistical Analysis

Analysis of variance (ANOVA) and significant difference among the means was used by applying Duncan's test (Reyes 1985) to the cholesterol-content results obtained from fresh shrimp to detect the internal variability in the raw sample. Also, a regression program (Number Cruncher Statistical Software [NCSS] Version 6.0.22, JMP, Version 3.1.2) was applied to the results obtained from the cholesterol-content analysis in the shrimp subjected

to freeze drying and supercritical extraction. Regression analysis allowed the application of a quadratic model which predicted the effect of the independent variables (pressure, CO_2 volume and temperature) on the residual cholesterol content in the shrimp. Because a central composite rotatory design was used, the confidence interval for the following model includes only the three central levels of each variable; the two outer levels were chosen ($2^{3/4} \times$ data radius) by the model to support the model validity within the desired range.

Thus, the model obtained is described by the equation

$$Y = B_0X_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{11}X_1^2 + B_{22}X_2^2 + B_{33}X_3^2 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + \varepsilon \quad (1)$$

where

X_1 = pressure 310 ± 21 with supporting values at $310 \pm 21 \times 2^{3/4}$
X_2 = CO_2 volume 1875 ± 966 with supporting values at $1875 \pm 966 \times 2^{3/4}$
X_3 = temperature 37 ± 1 with supporting values at $37 \pm 1 \times 2^{3/4}$

Graphs were constructed in three dimensions using the regression equation obtained. This was achieved using the STATISTICA Program (Version 4.5, 1993).

For the duo–trio test (sensory analysis), a Student *t*-test was used. The acceptance sensory analysis was performed using the nonparametric Kolmogorov-Smirnov test (NCSS Version 6.0.22, JMP, Version 3.1.2).

RESULTS AND DISCUSSION

Statistical Analysis

Duncan's Test (not shown) was used to determine a possible intrinsic variability in shrimp cholesterol content. The results yielded no significant differences in this parameter among all the fresh shrimp samples used for the experiment. The initial cholesterol content was 137 ± 11.15 mg/100 g on a wet basis.

Experimental Design

Table 1 shows the mean values obtained by GC for residual cholesterol in shrimp subjected to supercritical extraction. According to these results, several experimental runs yielded a product with a cholesterol content low enough to be classified as a low-cholesterol product, i.e., 100 mg or less per 100 g of shrimp on a dry weight basis.

TABLE 1.
EXPERIMENTAL VALUES FOR RESIDUAL CHOLESTEROL IN SHRIMP AS DETERMINED BY GAS CHROMATOGRAPHY

Treatment	X_1 P† (bar)	X_2 V‡§ (L)	X_3 T¶ (C)	Y Cholesterol (mg/100 g) dry basis
1	289 ± 2.45	909	36 ± 1.78	225.10 ± 17.20
2	331 ± 1.38	909	36 ± 1.31	292.71 ± 9.26
3	289 ± 1.69	2841	36 ± 2.30	151.41 ± 5.01
4	331 ± 2.02	2841	36 ± 2.01	81.96 ± 12.97
5	289 ± 2.84	909	38 ± 3.43	224.88 ± 4.40
6	331 ± 2.45	909	38 ± 2.66	211.06 ± 3.67
7	289 ± 1.19	2841	38 ± 3.54	72.19 ± 5.75
8	331 ± 1.35	2841	38 ± 2.95	52.02 ± 6.32
9	275 ± 1.23	1875	37 ± 2.09	114.25 ± 5.21
10	345 ± 1.01	1875	37 ± 2.36	99.23 ± 6.02
11	310 ± 1.45	250	37 ± 2.15	366.61 ± 3.23
12	310 ± 1.29	3500	37 ± 2.03	62.14 ± 7.28
13	310 ± 1.51	1875	35 ± 2.37	125.44 ± 4.30
14	310 ± 1.60	1875	39 ± 2.14	97.25 ± 3.52
15	310 ± 2.00	1875	37 ± 2.35	80.28 ± 7.87
16	310 ± 1.57	1875	37 ± 2.83	97.76 ± 9.44
17	310 ± 1.35	1875	37 ± 2.79	110.93 ± 7.11
18	310 ± 0.91	1875	37 ± 2.42	109.77 ± 5.04

† Pressure.
‡ CO_2 volume (measured at 1.013 bar and 25C).
§ The CO_2 volume is an established value for each run.
¶ Temperature.

The results were adjusted to a quadratic model using multiple regression analysis with the aim of generating an equation to predict the residual cholesterol content in supercritically extracted freeze-dried shrimp (Eq. 1). A significant effect ($P \leq 0.05$) was evident from the adjusted model, while the lack of adjustment was nonsignificant ($P > 0.05$). The correlation coefficient (R^2) was 0.9537.

According to the ANOVA performed ($P \leq 0.05$), quadratic and linear effects in the cholesterol-extraction operation were found. The most important factor was the quadratic effect of volume (Table 2). An additional effect from the pressure–volume factor was also noted, but this was found to be statistically nonsignificant ($P > 0.05$).

Figure 2 shows a response surface graph which illustrates the regression equation that was obtained. This graph shows the effect of the supercritical extraction operating conditions on the residual shrimp cholesterol content on

TABLE 2.
VALUE OF REGRESSION COEFFICIENTS CALCULATED FOR CHOLESTEROL EXTRACTION

Factor	Coefficient	*t*	Prob > |*t*|
Constant	6065.357	0.61	0.5587
Pressure	–0.6088331	–0.03	0.976724
Volume	0.1424819	0.36	0.729044
Temperature	–303.0457	–0.68	0.514805
Pressure–pressure	0.0147289	0.83	0.432685
Volume–volume	4.751141E-05	5.73	0.00041
Temperature–temperature	4.777326	0.84	0.425488
Pressure–volume	–8.837887E-04	–1.87	0.098741
Pressure–temperature	–0.1913458	–0.42	0.686505
Volume–temperature	–3.531664E-03	–0.36	0.731465

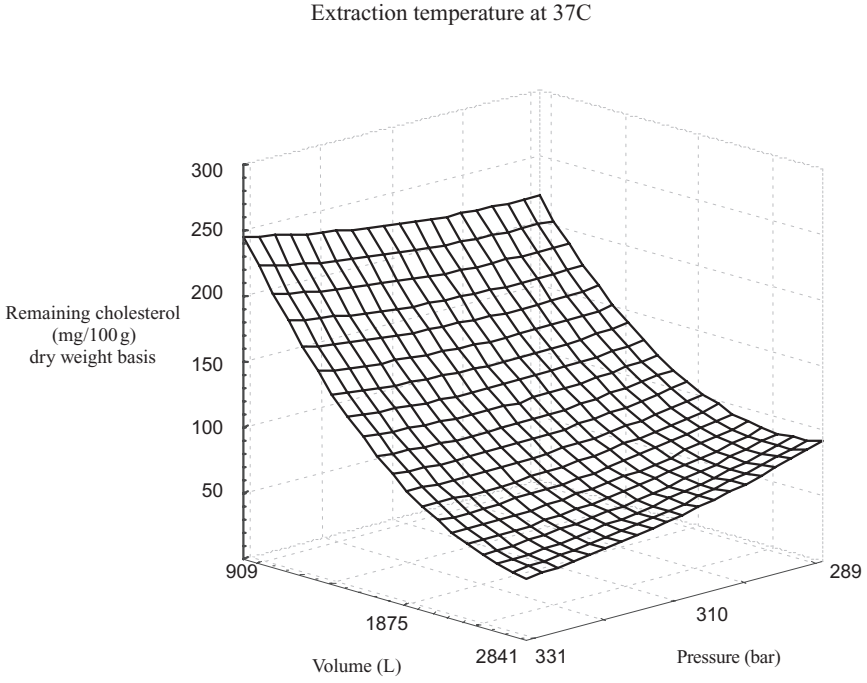

FIG. 2. SURFACE REPONSE GRAPH OF DIFFERENT CO_2 PRESSURE AND VOLUME OPERATING AT CONSTANT TEMPERATURE (37C)

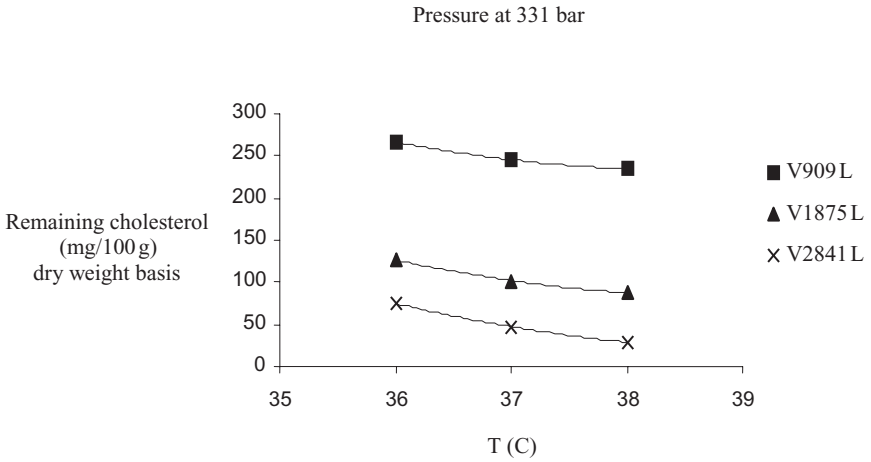

FIG. 3. EFFECT OF CO_2 TEMPERATURE AND VOLUME ON RESIDUAL CHOLESTEROL IN SHRIMP AFTER SUPERCRITICAL EXTRACTION AT 331 BAR PRESSURE

a dry weight basis. It presents the different extraction volumes required according to the final cholesterol content desired in shrimp.

Figure 3 shows the residual cholesterol content as a function of temperature for different CO_2 volumes at a fixed pressure of 331 bar. It can be observed that at such pressure, the residual cholesterol content decreases with an increasing CO_2 volume with respect to temperature. In a supercritical fluid, the effect of temperature on solubility is complex because of the interaction of two opposite and concurrent conditions, the increase in solubility and the decrease in density due to increasing temperature (Marentis 1988). In the experimental region used for this study, density is less sensitive to temperature changes while increases in vapor pressure becomes the dominant factor; therefore, an increase in temperature causes an increase in solubility. The temperature at which the lower residual cholesterol content is obtained is 38C (29.10 mg/100 g on a dry weight basis).

From Fig. 4, it can be observed that at 310 bar, 37C and 1875 L of CO_2, it is possible to obtain a mean value of 100 mg of residual cholesterol per 100 g of shrimp (on a dry weight basis). This is sufficient to obtain a final product which complies with the denomination of a low-cholesterol food (less than 24 mg of cholesterol/shrimp edible portion on a wet basis (FDA 1990). Figures 3 and 4 show that with other different combinations in operating conditions during the supercritical extraction, the same results can be obtained. However, the above-mentioned conditions are the less drastic ones, and this allows the minimization of the adverse consequences of the process.

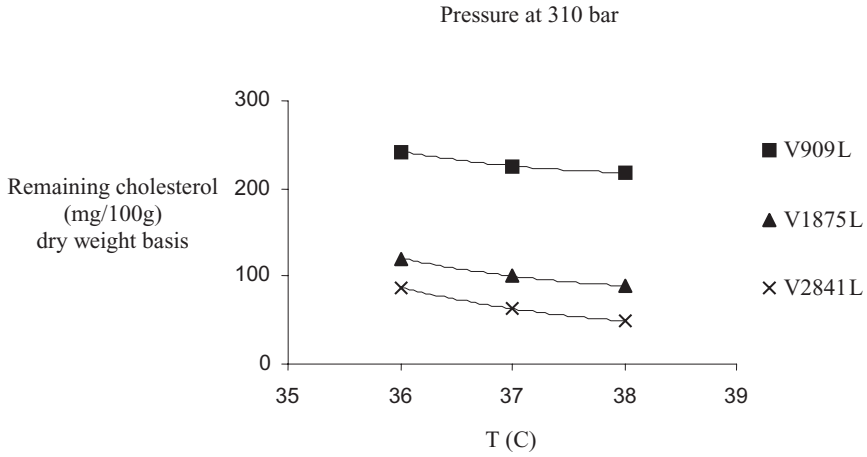

FIG. 4. EFFECT OF CO_2 TEMPERATURE AND VOLUME ON RESIDUAL CHOLESTEROL IN SHRIMP AFTER SUPERCRITICAL EXTRACTION AT 310 BAR PRESSURE

Sensory Analysis

Rehydration, Cooking and Sensory Evaluation. The initial rehydration tests in the freeze-dried shrimp were performed using a ratio of 1-g shrimp/3.6 mL of water at refrigeration temperatures (2–4C) for a 15-h period. Under these conditions, a rehydration index of 3.4 was obtained which was considered acceptable. An index of 4 is required for a moisture content equivalent to that of fresh shrimp. The rehydrated product was cooked in boiling water for 10 min using a ratio of 4-g shrimp/750 mL of water.

A sensory analysis was used to assess the effect of the freeze-drying process on the organoleptic properties of the rehydrated shrimp. A duo–trio test was used for this purpose. Two shrimp were served to each of the 30 members in the taste panel. They were asked to indicate which sample differed most regarding the control and to write down the difference in each of the five parameters: aroma, flavor, texture, color and overall appearance. A 5-point scale was used (from −2 to 2), and 33% of the panelists did not find differences between the freeze-dried shrimp and the fresh shrimp, while 66% perceived differences in color and texture. A statistical analysis by Student *t*-test did not show a significant difference in the parameters that were mentioned with the exception of texture ($P < 0.05$) which was less acceptable (−1) in freeze-dried shrimp versus the fresh one. Because of the differences in acceptability, several other cooking and rehydration procedures were tried to minimize the adverse

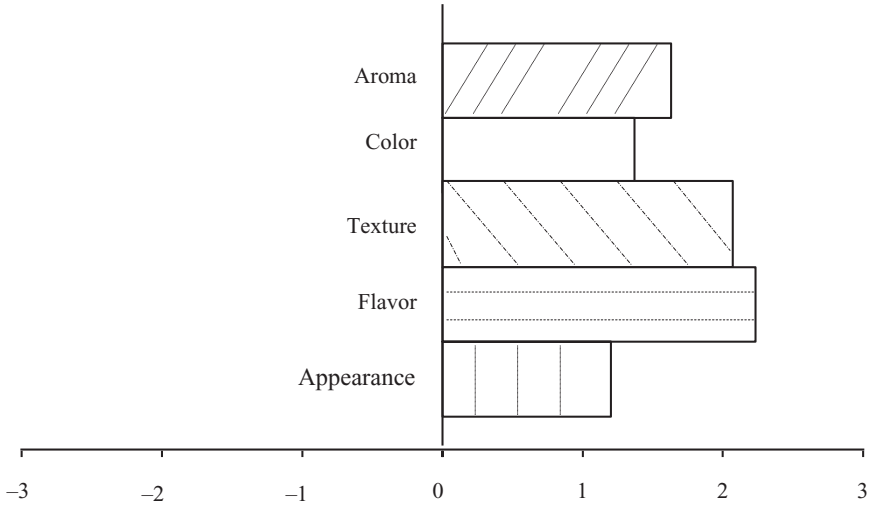

FIG. 5. ACCEPTABILITY TEST RESULTS FOR LOW-CHOLESTEROL SHRIMP
Dislike very much, –3; dislike moderately, –2; dislike slightly, –1; neither like nor dislike, 0; like slightly, +1; like moderately, +2; like very much, +3.

effect of processing on the freeze-dried product characteristics and also on the shrimp subjected to freeze drying and supercritical extraction.

A method for standardizing rehydration under vacuum (21″ Hg) at room temperature was developed. The shrimp were placed in a vacuum chamber for 2 h and overturned after half the period had elapsed. A ratio of 5-mL water/g of shrimp was used. A rehydration index of 3.6 was obtained for the freeze-dried shrimp that underwent supercritical extraction. Upon rehydration, the shrimp were steam cooked.

An acceptability test using a 30-member untrained panel was performed whereby the acceptability, aroma, flavor, texture and color of the rehydrated low-cholesterol shrimp were assessed. The assessment scale had 7 points (from –3 to +3). For the statistical analysis, a nonparametric Kolmogorov–Smirnov test was used. All attributes were assessed positively by the panel members (Fig. 5), and no significant differences were found for the overall acceptability attribute. These results clearly pointed to the fact that rehydration under vacuum and steam cooking had a significant improvement on the texture, aroma and overall acceptability of the shrimp subjected to freeze drying and supercritical extraction.

Economic Feasibility of the Process

The economic feasibility of supercritical extraction for producing a low-cholesterol shrimp will depend mainly on the price of the raw material and the

initial capital investment which is dependent upon the level of production desired. The market for low-cholesterol foods is increasing and should prove to be a major positive factor in a commercial venture.

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