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The influences of cultivar and thermal processing on the allergenic potency of lychees (*Litchi chinensis* SONN.)

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Abstract

This study was aimed at the cultivar-specific allergenic potency of lychees (*Litchi chinensis* SONN.) and its modification by typical industrial processing, investigating the fresh aril of seven different lychee lots which represented five cultivars. Technological focus was on thermal treatments during fruit preservation by canning. Canned lychee halves in syrup were produced by canning at 90 and 121 °C for various times to analyse the final products immediately after processing and after eight-months storage. SDS–PAGE and non-specific silver staining were performed to characterise the protein pattern. The allergenic potency of the proteins was demonstrated by immunoblotting with sera of probands suffering from lychee allergy. Furthermore, the allergenic potency was determined by inhibitive enzyme allergosorbent tests (EAST-inhibition). Any significant dependence between cultivar and the allergenic potency of the fresh fruit could not be established. According to their heat sensitivity during canning, lychee allergens with different behaviour could be distinguished. After excessive canning, inducing severe loss of sensory quality, the allergenic potency of the fruit decreased, even though high residual allergenic activity was observed.

Keywords: *Litchi chinensis* SONN.; Food allergy; Cultivar; Canning; Sensory quality

1. Introduction

Owing to the improved transport conditions, the consumption of tropical fruits has increasingly gained significance in the Western diets, in parallel with the continual rise of their annual world production to approx 14.5×10^6 Mt (FAO, 2004) in 2003. *Litchi chinensis* SONN. is a non-climacteric fruit cultivated in many subtropical areas around the world (Kadam & Deshpande, 1995). In accordance with their main utilisation as fresh or canned fruits, most studies on the use of lychees have been focused on the description of cultivars with respect to nutritional aspects (Kadam & Deshpande, 1995; Menzel & Simpson, 1991), the discolouration of the pericarp during marketing of fresh fruits (Holcroft & Mitcham, 1996; Ray, 1998; Sarni-Manchado, Le Roux, Le Guernevé, Lozano, & Cheynier, 2000; Underhill & Critchley, 1995) and the pink discolouration of the fruit flesh observed on canning (Wu & Fang, 1993; Wu & Sheu, 1996). With respect to increasing export markets, recent research has primarily focused on postharvest biology and technology of lychee fruits, aimed at the production of light-coloured, chemical-free fruits without disease or insect infestation (Jiang, Yao, Lichter,

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& Li, 2003). The nutritional value of lychees is based on the vitamin C content of its edible aril, which is 40–90 mg/100 g (Kadam & Deshpande, 1995). Depending on cultivar, cultivation area, storage and processing conditions, the vitamin C content is subjected to strong variations and less significant vitamin C levels were also reported (Ramma, Bahorun, & Crozier, 2003).

Reports of adverse reactions to lychee list symptoms such as urticaria, erythema (Montag, Kalveram, & Gall, 1996), pruritus, swelling of lips and fingers, dyspnoea and diarrhoea (Leep, Uhlemann, Beck, & Schlaak, 1992). Fäh, Wüthrich, and Vieths (1995) and Niggemann, Reibel, Hipler, and Wahn (2002) even reported of patients with anaphylactic shock reactions after eating lychees. Giannattasio et al. (1995) presented a case report on a 34-year-old woman suffering from generalised urticaria, Quincke oedema and bronchospasm after eating lychee fruits.

About 2% of adults and up to 6–8% of children suffer from food allergies (Fogg & Spergel, 2003; Sampson, 2003). The allergenic potency of lychee may depend on its cultivar and may be altered during processing. According to Vieths, Schöning, and Jankiewicz (1993) and Vieths, Jankiewicz, Schöning, and Aulepp (1994), the allergenic potency of apples depends on their cultivar. The allergenic activity could remain unchanged during processing (Dube et al., 2004), but it could be also decreased, e.g., by thermal deformation of allergenic structures. The molecular basis of changes in the allergenic activity may be the inactivation or destruction of epitope structures, the formation of new epitopes or better access of hidden epitopes by denaturation of the native allergen. Various technological procedures in food processing may affect the proteins in the food and hence allergenic potency. The principal molecular and functional characteristics of food allergens from plant origin have been recently reviewed (Mills, Madsen, Shewry, & Wichers, 2003). Allergy to lychee has been reported for a long time to be caused by cross-reactive structures after sensitisation by inhalent allergens of pollen (Fäh et al., 1995), especially from birch (Wellhausen, Schöning, Petersen, & Vieths, 1996). As in many other fruits and vegetables, IgE-binding proteins related to the birch pollen minor allergen Bet v 6 were found in lychee fruits (Vieths, Scheurer, & Ballmer-Weber, 2002). Generally, pollen-related allergens are known to be more labile during heating and in the digestive tract compared to the allergens comprising the 7S and 11S globulin storage proteins of the cupin superfamily (Mills et al., 2003; Vieths et al., 2002).

Considering the principal use of lychees, this study was aimed at the impacts of cultivar and conventional canning procedures on their allergenic potency. Focus was on the allergen pattern in lychee fruits and on the verification of the hypothesis that usual heat treatments applied in industrial fruit processing could sufficiently inactivate the lychee allergens. In an interdisciplinary approach, the influence of heating on the allergenic potency was contrasted with the thermal effects on sensory product properties such as firmness and colour.

2. Materials and methods

2.1. Origin of fruits

To explore the impact of the cultivar on the allergenic potency, fresh fruits of seven lychee lots (edible part) were analysed by electrophoresis, immunoblotting and EAST-inhibition. For this investigation, the varieties 'Kuang Chao', 'Bai Dum', 'Chacapat', and 'Hong Huey' from Thailand as well as 'Mauritius' and 'Cope' from South Africa and one unknown variety from Madagaskar were chosen. The fruits representing the four Thai cultivars were grown in Chiang Mai Province. Directly after harvest, they were obtained from local orchards and sent to the University of Hamburg by airfreight. Fruits were cooled by means of dry ice during the transport and immediately analysed after their arrival. The other cultivars were obtained from local wholesalers in Stuttgart and Hamburg, respectively. For canning experiments, fresh fruits of the unknown cultivar from Madagaskar mentioned above were obtained from the local wholsaler in Stuttgart and immediately used for processing.

2.2. Lychee processing

Canned lychees in syrup were produced at the pilot plant scale at Hohenheim University according to the German standard specifications for processed fruits (Deutsche Lebensmittelbuch-Kommission, 2004). Lot size per variant was 4.5 and 5 kg fruit for V1–V4 and V5–V9, respectively. Apart from water, saccharose and citric acid, no other ingredients were added. The saccharose concentration of the infusion liquid at filling (S_a in g/100 g) was calculated based on the total soluble solids (TSS) of the processed raw material (r in °Brix, i.e., in g saccharose/100 g) and the target specification (S_f = target saccharose concentration of the final product in g/100 g after concentration balance, S_f = 17 °Brix) (Eq. (1)). To adjust the pH in the product, the necessary concentration of citric acid in the added infusion liquid was determined by titration of a ground aril sample with 1 M citric acid to the target pH after dilution of this sample according to the ratio of fruit net weight F and total net weight G

$$S_a = (F \cdot (S_f - r)/(G - F)) + S_f, \quad (1)$$

with F is the fruit net weight (g) and G is the total net weight of fruit and infusion liquid (g).

Washed and inspected fruits were manually peeled, destoned and cut into halves. Aril halves were filled into coated aluminium cans (83 × 85.6 mm) prior to adding the infusion liquid mentioned above until the standardised filling volume (50 g fruit/100 mL for destoned and peeled fruits). During sealing of the cans, using a semi-automatic vacuum-sealing machine VCV 357 (Maschinenfabrik Clemens & Vogl, Braunschweig, Germany), a vacuum of 300 mbar was generated to minimise oxidative quality loss. Pasteurisation (variants V1–V8) and sterilisation (V9) at 90 and 121 °C, respectively (Table 1), was performed in an autoclave type Stock Pilot-Rotor 900 (Satori Stocktec GmbH, Neumünster, Germany) in the sprinkling mode under rotation of the cans at 7 rpm. To control the efficiency of pasteurisation and sterilisation, respectively, three cans of each lot were used for the recording of internal temperatures at intervals of 1 min during heating, holding at target temperature and cooling to an internal temperature ⩽20 °C, after placing a temperature sensor in the infusion liquid in the thermal centre of each can. The cans were stored at 4 °C for 24 h for the concentration balance between fruit and infusion liquid prior to analysis of the final products. Cans of the variants V1–V4 (mild pasteurisation) were also investigated after storage of eight months at 4 °C to assess potential alterations during storage.

The heating effects which the aril halves were exposed to during the entire pasteurisation and sterilisation processes, respectively, are described by both the microbial

Table 1
Production of canned lychees in syrup (coated aluminium cans, 83 × 85.6 mm, 420 g total net weight): process parameters and main characteristics of resulting product quality

Variant	V1	V2	V3	V4	V5	V6	V7	V8	V9
Process parameters									
Adjustment of pH to…	pH 3.5					4.0 < pH < 4.5			
A. Hygenic heating concept									
Nominal pasteurisation time (*P*-value), $P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ [min]	0.9 ± 0.07[a]	2.2 ± 0.11	3.5 ± 0.25	5.8 ± 0.13	6.0 ± 0.04	10.1 ± 0.06	15.2 ± 0.08	19.4 ± 0.03	–
Nominal sterilisation time (*F*-value), $F^{z=10\,^{\circ}C}_{T_{ref}=121.1\,^{\circ}C}$ [min]	–	–	–	–	–	–	–	–	12.4 ± 0.23
Max internal temperature, $T_{c,max}$ [°C]	88.6	90.1	90.5	90.9	90.5	90.7	91.0	90.8	121.5
Max autoclave temperature, $T_{a,max}$ [°C]	90–91	90–91	90–91	90–91	90–91	90–91	90–91	90–91	120–121
Holding time at $T_{a,max}$ [min]	3	6	10	13	12	20	30	40	12
B. Nominal cooking damage									
C-value, $C^{z'=25\,^{\circ}C}_{T_{ref}=100\,^{\circ}C}$ [min]	1.8 ± 0.07	3.0 ± 0.06	4.4 ± 0.15	6.1 ± 0.04	6.5 ± 0.03	9.7 ± 0.03	13.9 ± 0.04	17.7 ± 0.04	102.7 ± 1.35
Quality characteristics									
A. Raw lychee aril									
Cultivar	Not specified						cv 'Mauritius'		
TSS[b] [°Brix]	17.3						18.0		
B. Product after 24 h at 4 °C									
Drip weight [g/100 g total net weight]	57.6	56.7	58.8	58.2	57.0	54.9	55.3	56.8	55.0
pH	3.8	3.7	4.3	4.2	4.2	4.2	4.2	4.2	4.2
TSS [°Brix]	16.8	17.1	16.9	17.3	18.0	17.2	17.8	18.1	18.0
TA[c] (pH 8.1, citric acid) [g/kg]	4.4	4.4	3.0	3.1	2.7	2.8	2.6	2.4	2.5
C. Product after 8 months at 4 °C									
pH	3.8	3.6	4.3	4.2	na[d]	na	na	na	na
TSS [°Brix]	18.0	18.1	18.0	17.9	na	na	na	na	na
TA (pH 8.1, citric acid) [g/kg]	4.2	5.3	2.5	2.9	na	na	na	na	na

[a] Mean ± standard error.
[b] TSS, total soluble solids.
[c] TA, titratable acids.
[d] na, not analyzed.

process lethality in the can (pasteurisation value $P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ and sterilisation value $F^{z=10\,^{\circ}C}_{T_{ref}=121.1\,^{\circ}C}$, respectively) according to Eqs. (2) and (3) and the index for sensory degradation (cooking value $C^{z'=25\,^{\circ}C}_{T_{ref}=100\,^{\circ}C}$) defined by Eq. (4) (Table 1; Larousse & Brown, 1997)

$$\text{Pasteurisation value: } P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C} = \int_0^t 10^{(T(t)-T_{ref})/z}\,dt. \quad (2)$$

$$\text{Sterilisation value: } F^{z=10\,^{\circ}C}_{T_{ref}=121.1\,^{\circ}C} = \int_0^t 10^{(T(t)-T_{ref})/z}\,dt. \quad (3)$$

$$\text{Cooking value: } C^{z'=25\,^{\circ}C}_{T_{ref}=100\,^{\circ}C} = \int_0^t 10^{(T(t)-T_{ref})/z'}\,dt. \quad (4)$$

The P- and F-values, respectively, express the duration of the treatment at the reference temperature T_{ref} that has the same effect on a population of microorganisms, which is characteristic for the product, as the treatment for a time t at varying temperatures T throughout the process. Thus the effects of heating and cooling phases of a process are included. The z-value depends upon the microorganism under consideration and the nature of the product. The general equation defining the cooking value was developed by analogy. Reference temperatures as well as z- and z'-values used in the present application (pH < 4.5) were chosen as indicated in Eqs. (2)–(4) (Brown, 1991; Larousse & Brown, 1997).

2.3. Control of fruit and product quality

In process control, the raw and canned lychee halves, respectively, were homogenised in a laboratory blender Moulinex D56 (Groupe SEB, Ecully, France) before determining total soluble solids (TSS), titratable acidity (TA), pH and colour. TSS was measured refractometrically (IFU, 2001). TA was determined by titration with 0.1 M NaOH to a pH of 8.1 and expressed as g citric acid/100 g (IFU, 2001). Aril colour was evaluated with a colorimeter CR300 (Minolta, Tokyo, Japan) based on the CIELAB colour system (L^*, a^*, b^* and L^*, C^*, H°, respectively) (Hutchings, 1999), measured from a freshly obtained purée, which was homogeneously distributed on a Petri-plate placed on a white background. Colour data sets were measured 10-fold per sample. Characterising colour intensity, chroma $C^* = ((a^*)^2 + (b^*)^2)^{0.5}$ describes the length of the colour vector in the rectangular $a^* \times b^*$-space, where a^* and b^* specify the green-red and the blue-yellow hue axes, respectively, ranging from a^- (green) to a^+ (red) and from b^- (blue) to b^+ (yellow). The hue angle $H^\circ = (180^\circ/\pi) \cdot \arctan(b^*/a^*)$, in this study in degree, marks the position of this vector and hence the nature of the hue with $H^\circ = 0$ and $H^\circ = 90^\circ$ for pure red and yellow, respectively. The lightness L^* completes this coordinate system to the three-dimensional colour space. In order to evaluate aril firmness, the specific maximum force (F_{max} in N/100 g), which was needed to crush a sample of 40 ± 0.2 g lychee halves in the Kramer shear cell by means of shear, compression and extrusion (Harker, Redgwell, Hallett, & Murray, 1997), was measured at a crosshead speed of 100 mm/min, using an Instron Universal Texture Analyzer 4301 (Instron, Canton, MA, USA) equipped with a 5-kN load cell. At least five measurements with new fillings of the shear cell were carried out per sample. Following the standard procedure for the determination of product filling volumes (Nehring, Nehring, Hanebuth, & Demarrez, 1996), the drip weight of the preserved fruits was determined gravimetrically, after the syrup had drained off within exactly 2 min from a sieve with 2.8 mm mesh size.

2.4. Patients and human sera

Immunological investigations such as immunoblot and EAST-inhibition assays were performed at the University of Hamburg, using a pool-serum consisting of the sera from 15 patients who suffered from adverse reactions to lychee (Table 2). All sera were collected at the University Hospital Eppendorf, Department of Dermatology and Allergy, Hamburg, Germany. Sera from non-atopic patients were used as negative control. Specific IgE in the sera was determined by an enzyme allergosorbent test (EAST) using lychee protein extract coupled to cyanogen bromide activated filter paper disks according to the manufacturer's instructions (Allergopharma, Reinbek, Germany) (Table 2).

Table 2
Specification of the patients' sera

No.	Patient	Sex	EAST-class/Spec. IgE [U/mL]	Clinical symptoms
1	RD	m	3/3.93	IM, RH
2	RF	f	2/1.84	IM, SL
3	CF	f	2/0.93	–
4	KDH	m	4/>17.5	RH, E, D
5	UH	f	2/1.04	ST, B
6	RK	m	3/5.72	SA, S
7	SK	f	3/10.1	ST, B
8	KL	m	2/0.71	–
9	CL	f	2/0.69	D, SL, IM
10	FR	m	3/17.3	IM, SL
11	LS	m	3/4.27	–
12	FS	m	3/4.27	SL, ST, IM
13	DS	f	4/>17.5	ST, IM
14	SW	m	2/3.30	–
15	UW	f	3/9.50	IM

Clinical symptoms: B, breathlessness; D, dermatitis; E, eczema; IM, itching in mouth; RH, rhinitis; S, sickness; SA, stomach ache; SL, swelling of lips; SM, swelling in mouth; ST, swelling of throat.
m, male; f, female.

2.5. Preparation and electrophoretic characterisation of allergen extracts

Lychee protein extracts were prepared from the edible part (aril) of fresh and canned lychees by a low-temperature method described previously (Möller et al., 1997; Vieths, Schöning, & Baltes, 1992). Proteins were separated by SDS–PAGE in 10% Bis–Tris Gels (Invitrogen, Groningen, Netherlands) according to the manufacturer's instructions. The electrophoretically separated proteins were visualised by silver staining (Heuckeshoven & Dernick, 1986) on the one side and transferred onto Protran nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany) by semidry blotting (Kyhse-Andersen, 1984) with a discontinuous buffer system (Vieths et al., 1992) on the other side. The molecular weights were determined using a protein standard mixture (14.4–94.0 kDa) after silver staining or, on nitrocellulose membranes, after gold-staining (BioRad, Munich, Germany).

2.6. Immunological characterisation of the allergenic potency

For immunoblotting, the nitrocellulose membranes were incubated with the pooled sera (diluted 1:15) mentioned above. Sera from non-atopic patients were used as negative control. Immuno-staining was performed according to Vieths et al. (1992) and Möller et al. (1997).

EAST-inhibition of IgE-binding to lychee proteins was performed using extracts of the fresh lychee aril (cultivar as indicated below for the respective experiment), which were coupled to bromocyan-activated cellulose paper disks. The disks were incubated with sera and inhibitor extracts. Extracts of fresh aril from different lychee varieties and of canned lychee halves were used as inhibitors in comparisons of cultivars and product variants, respectively.

3. Results

3.1. Allergenic potency of lychee cultivars

After protein separation in the fresh aril extracts of seven different lychee lots by SDS–PAGE, several IgE-binding protein bands were identified in a molecular weight range from 14 to 94 kDa by immunoblotting. The protein extracts obtained from different varieties did not significantly differ in SDS–PAGE after silver staining (one example is depicted in Fig. 1) and immunoblotting (Fig. 1). In each extract, IgE-binding proteins with molecular weights of 14, 25, 28, 32, 35, 40, 43, 55, 60, 65 and 70 kDa were detected. Additionally, several IgE-binding proteins with molecular weights >70 kDa were stained weakly. The allergens being particularly noteworthy had molecular weights of 14, 35, 40, 43, 55 and 70 kDa. Allergens with an approximate molecular weight of 25 and 28 kDa, respectively, were observed with varying intensity depending on the cultivar. Nevertheless, owing to the similar pattern of IgE-binding proteins in all extracts both with respect to molecular weight and intensity, cultivar-specific differences in the allergenic potency of lychee fruits could not be found.

The immunological efficiency of the allergen patterns shown by immunoblot studies was investigated by means of EAST-inhibition assays. Aimed at the relative quantification of the allergenic potency of each cultivar,

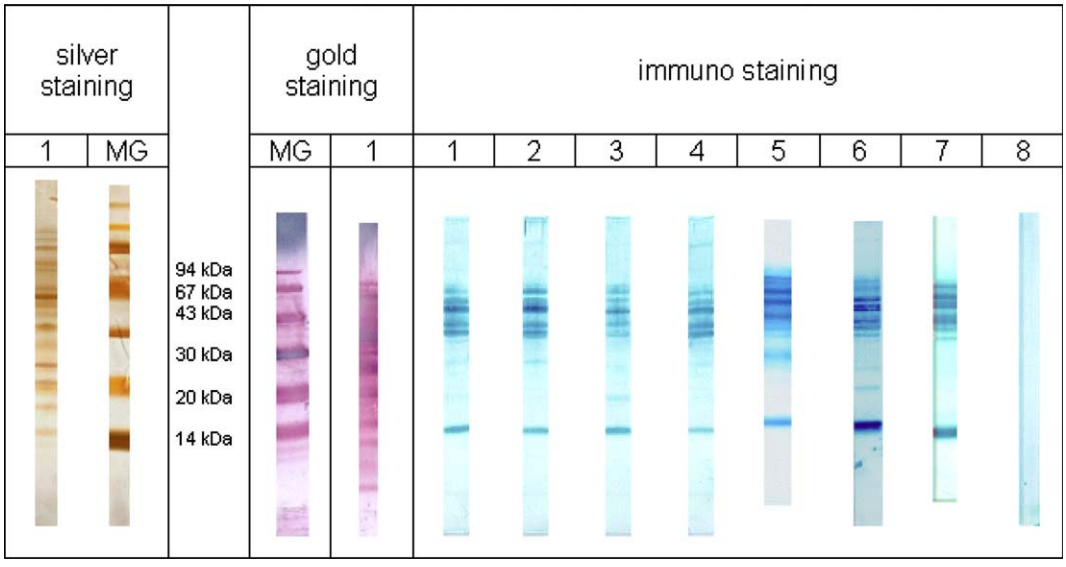

Fig. 1. Immunoblot of extracts from the fresh aril of different lychee cultivars. 1: cv 'Chacapat'; 2: cv 'Bai Dum'; 3: cv 'Kuang Chao'; 4: cv 'Hong Huey'; 5: cv 'Mauritius'; 6: cv 'Cope'; 7: unspecified cultivar; 8: negative control; MG: molecular mass marker.

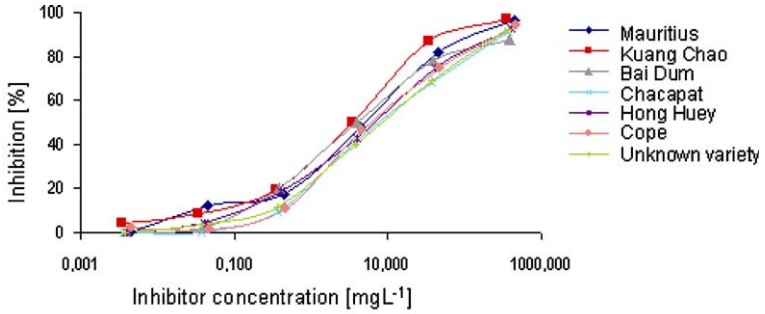

Fig. 2. EAST-inhibition of the allergens of fresh lychee aril cv 'Mauritius' by extracts of various lychee cultivars.

the respective extract of the fresh aril was bound to a solid phase as reference, while the extracts of the other six fruit lots were used as inhibitors, each in form of a concentration series. Control was the extract of the same fruit lot as unbound inhibitor extract for homologue inhibition. Plotting relative inhibition of the immobilised allergenic proteins against the inhibitor concentration (Fig. 2), graphs were generated visualising the inhibiting effects among the cultivars for each reference extract. To facilitate comparison, each fruit lot was characterised by its extract concentration resulting in 50% inhibition of the bound reference allergens (C_{50}-value in mg/L). In this multiple approach, using each cultivar-specific protein extract once as immobilised reference, comparable graphs were generated. In Fig. 2, EAST-inhibition of the cultivar 'Mauritius' by all cultivar-specific extracts is exemplarily shown, revealing similar graphs for all variants. Irrespective of the chosen reference, the C_{50}-values of each fruit lot were comparable. As an example, C_{50}-values for inhibition of immobilised proteins of the cultivar 'Mauritius' are summarised in Table 3. Ranging from 3.4 to 8.4 mg/L, the C_{50}-values of all fruit lots were very close to the value of the reference cultivar 'Mauritius' (5.2 mg/L). Thus, the studied lychee cultivars did not differ in their allergenic potency, confirming the uniform allergen pattern found by immunoblotting for fresh lychee aril.

Table 3
Allergenic potency of various lychee cultivars

Lychee cultivar	C_{50}-value [mg/L]
Homologue inhibition	5.2
Kuang Chao	7.9
Bai Dum	6.8
Chacapat	3.9
Hong Huey	6.4
Cope	8.4
Unspecified cultivar	3.4

Their extracts were used as inhibitors in EAST-inhibition experiments. C_{50}-values obtained by inhibiting bound allergens of fresh lychees cv 'Mauritius'.

3.2. Impact of heat treatments applied in canning on the sensory quality of lychees

Consistent with the high TSS content of fully ripe fruits after harvest, canned lychees are mainly offered in the class of 17–20 °Brix in the final product according to the German standard specifications for processed fruits (Deutsche Lebensmittelbuch-Kommission, 2004). When preserving lychees in the standardised form (whole fruit without seed and peel), the cans are characterised by a drip weight of 50 g/100 mL (Nehring et al., 1996). Regarding TSS of raw material and products as well as the drip weight of the samples used in this allergological study (Table 1), the pilot plant products represented the typical nature of the target product group. Because of the close adjustment of the sugar concentration in the syrup to fruit TSS, osmotic alterations between fruit and syrup were minimised as shown by the TSS of the fruit before canning as well as 24 h and 8 months after processing. Thus, tissue damage was restricted to the new surfaces after cutting the aril into halves.

Microbial lethality ($P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ and $F^{z=10\,^{\circ}C}_{T_{ref}=121.1\,^{\circ}C}$ values, respectively) and sensory damage ($C^{z'=25\,^{\circ}C}_{T_{ref}=100\,^{\circ}C}$ value) induced by the heating treatments increased from variant V1 to V9 (Table 1). Concerning pasteurisation, the $C^{z'=25\,^{\circ}C}_{T_{ref}=100\,^{\circ}C}$ value reached in variant V8 was approx 10-fold of that in V1. Sterilisation of variant V9 caused a cooking damage, which was approximately 6 times higher than that induced by the strongest pasteurisation treatment (V8). With respect to product quality, texture and colour were characterised, since these properties determine sensory quality and product appearance regarding consumers' acceptance most strikingly. Pasteurisation up to a $P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ value of 10 min did not affect the aril firmness measured 24 h after production (Table 4). After storage for eight months, no or only very slight softening was observed for $P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ values of 0.9–2.2 and 3.5–5.8 min, respectively. Above $P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ = 15 min (variant V7), softening of the canned fruits was increasingly noticed. When compared to the raw fruit, relative firmness reduction was 37% after pasteurisation at $P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ = 19 min (V8) and

Table 4
Effects of pasteurisation (V1–V8) and sterilisation (V9) of lychee fruit halves on their allergenic potency, firmness and colour

	Storage of cans (4 °C)	Variant								
		V1	V2	V3	V4	V5	V6	V7	V8	V9
C_{50}-value [mg/L]	24 h	2.3	5.9	3.2	13.3	9.1	39.8	45.0	39.2	75.5
	8 months	28.8	6.7	2.5	5.0	na	na	na	na	na
Firmness										
F_{max} [N/100 g]	24 h	2653 ± 73 (+20.7%)	2357 ± 98 (+7.2%)	2298 ± 64 (+4.5%)	2273 ± 15 (+3.4%)	1835 ± 77 (+14.8%)	1475 ± 64 (−7.7%)	1313 ± 47 (−17.8%)	1005 ± 24 (−37.1%)	334 ± 12 (−79.1%)
	8 months	2843 ± 44 (+29.3%)	2193 ± 43 (−0.2%)	2051 ± 55 (−6.7%)	1941 ± 136 (−11.7%)	na	na	na	na	na
Colour										
Lightness L^*	24 h	64.9 ± 0.44	68.0 ± 0.29	68.8 ± 0.10	74.8 ± 0.18	79.3 ± 0.33	79.3 ± 0.15	78.4 ± 0.19	71.4 ± 0.22	63.4 ± 0.21
	8 months	66.2 ± 0.29	64.1 ± 0.53	68.4 ± 0.25	63.5 ± 0.64	na	na	na	na	na
Redness a^*	24 h	3.6 ± 0.18	3.3 ± 0.07	2.5 ± 0.04	2.1 ± 0.03	0.2 ± 0.02	0.5 ± 0.02	0.9 ± 0.02	0.4 ± 0.09	11.8 ± 0.12
	8 months	2.6 ± 0.06	3.1 ± 0.10	1.7 ± 0.06	3.4 ± 0.15	na	na	na	na	na
Yellowness b^*	24 h	6.0 ± 0.31	7.6 ± 0.22	6.9 ± 0.09	8.2 ± 0.09	6.3 ± 0.07	6.6 ± 0.08	7.4 ± 0.12	7.4 ± 0.17	13.2 ± 0.12
	8 months	8.0 ± 0.10	7.5 ± 0.25	6.9 ± 0.30	7.1 ± 0.32	na	na	na	na	na
Hue angle H_{ab} [°]	24 h	59.3 (−11.4%)	66.5 (−0.6%)	69.9 (+4.5%)	76.0 (+13.6%)	88.3 (−9.8%)	85.7 (−12.3%)	82.8 (−15.3%)	86.8 (−11.3%)	48.2 (−50.8%)
	8 months	71.9 (+7.4%)	67.5 (+0.8%)	76.4 (+14.1%)	64.4 (−3.8%)	na	na	na	na	na
Chroma C_{ab}	24 h	7.0 (−17.7%)	8.3 (−1.7%)	7.4 (−12.8%)	8.5 (+0.4%)	6.3 (+44.1%)	6.6 (+51.8%)	7.5 (+71.8%)	7.4 (+71.2%)	17.7 (+307%)
	8 months	8.4 (−0.8%)	8.1 (−3.9%)	7.1 (−15.5%)	7.9 (−6.8%)	na	na	na	na	na

C_{50}-values of canned lychees were obtained by EAST-inhibition experiments, inhibiting the bound allergens of the fresh lychee by the active allergens of the canned fruits (C_{50}-value of homologue inhibition: 13.9 mg/L). Relative changes of firmness, hue and chroma, respectively, are referred to the processed raw material and are indicated in parentheses.
na, not analyzed; values ± standard error.

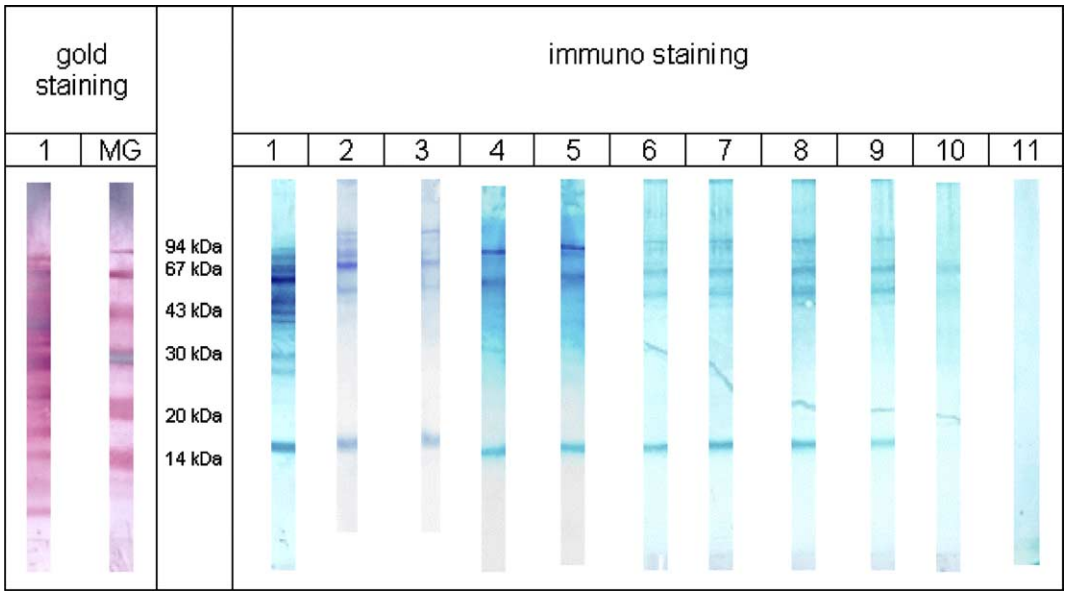

Fig. 3. Immunoblot of canned lychees. 1: native lychee; 2–10: product variants V1–V9; 11: negative control; MG: molecular mass marker.

even 79% after sterilisation at $F^{z=10\,^{\circ}C}_{T_{ref}=121.1\,^{\circ}C}$ = 12.4 min (V9). As revealed by the colour components describing lightness, redness and yellowness in Table 4 (L^*, a^* and b^*, respectively), aril colour was less affected by heating than firmness. Only sterilisation at $F^{z=10\,^{\circ}C}_{T_{ref}=121.1\,^{\circ}C}$ = 12.4 min (variant V9) induced pronounced pink discolouration indicated by a tremendous increase of redness from $a^* = -0.6$ in the greenish-white raw material to 11.8 in the yellowish-pink canned fruit. Consistently, the inacceptable change in the entire hue induced by this sterilisation process became evident in the massive reduction of the hue angle H° by 50.75% and the increase in colour intensity marked by the 4 times higher chroma C^*. Contrarily, measurable alterations from greenish-white towards reddish-white induced by pasteurisation in variants V5–V8 ($P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ = 6–19 min) were only hardly visible and thus not of sensory relevance. Moreover, the canned fruits of these variants possessed a much purer white colour than variants V1–V4 resulting from milder pasteurisation, because the latter four variants were produced from a raw material lot which already showed a rather impure pink-white hue. In conclusion, sensory quality was affected by pasteurisation above $P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ = 15 min (variant V7), resulting in hardly acceptable and even inacceptable products after pasteurisation at $P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ = 19 min (V8) and sterilisation at $F^{z=10\,^{\circ}C}_{T_{ref}=121.1\,^{\circ}C}$ = 12.4 min (V9), respectively.

3.3. Impact of heat treatments applied in canning on the allergenic potency of lychees

Alterations in the allergen pattern of lychee fruits thermally induced by canning were studied by immunoblotting (Fig. 3). The protein extracts obtained from lychee products V1–V8, which had been subjected to increasing pasteurisation effects, did not show any significant differences in the allergen pattern among each other. When compared to the extract of the unheated fruit, the characteristic IgE-binding proteins with molecular weights of 14, 40, 43, 55 kDa, 70 and approximately 94 kDa were also observed after all pasteurisation treatments applied. However, the IgE-binding proteins with molecular weights of 25, 28, 32, and 35 kDa were no longer detectable. After sterilisation (V9), only one IgE-binding protein with a molecular weight of 55 kDa could be stained. In conclusion, the lychee allergens could be classified into three groups of heat sensitivity. After storage of the cans V1–V4 for eight months, the same IgE-binding proteins were detected (Fig. 4) as those in cans directly after production.

To assess the allergenic potency of canned lychees by EAST-inhibition, an extract of unheated lychee aril from the raw material lot used in processing was bound to a solid phase, while the protein extracts of the canned fruits (after 24 h and 8 months, respectively) were used as inhibitors. Control was the extract of the raw material applied for homologue inhibition (Table 4: C_{50}-value 13.9 mg/L). The C_{50}-values of the protein extracts gained from variants V1 to V5 (Table 4: 2.3–9.1 mg/L) did not differ from the C_{50}-values of the unheated fruit (Table 3: 3.4–8.4 mg/L) as well as the homologue inhibition shown in Table 4 and hence in their allergenic potency. Variant V1, storage 8 months with a C_{50} value of 28.8 mg/L has also no statistical relevance. Thus, thermal treatments of the lychee halves equivalent to heating up to $P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ = 6 min did not affect the allergenic potency of lychees. Enhanced pasteurisation

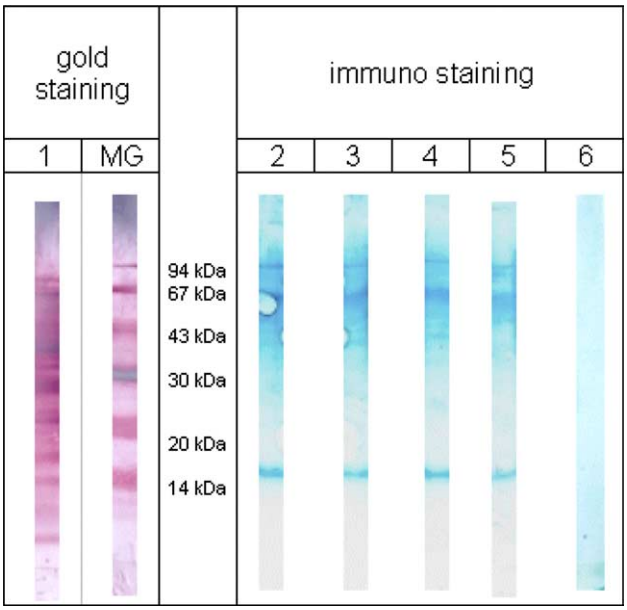

Fig. 4. Immunoblot of canned lychees after eight-months storage. 1: native lychee; 2–5: product variants V1–V4 after storage; 6: negative control; MG: molecular mass marker.

($P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ = 10–19 min, variants V6–V8) and extreme sterilisation at $F^{z=10\,^{\circ}C}_{T_{ref}=121.1\,^{\circ}C}$ = 12.4 min (variant V9) resulted in a slight decrease of the allergenic potency as displayed by rising C_{50}-values (Table 4), irrespective of the differences in the allergen pattern between V6–V8 and V9. After sterilisation of variant V9, the C_{50}-value was only 5.4 times higher than that of the fresh fruit extract. The C_{50}-values of the pasteurised variants V6–V8 were even only at the 2.8–3.2-fold level. The C_{50}-values were confirmed within their natural variation range by analogous investigations after a storage of the cans of variants V1–V4 for 8 months (Table 4). On the whole, the allergen pattern revealed by immunoblotting could be markedly influenced by the applied canning treatments, but heating could hardly reduce the allergenic potency of the fruits. The existence of a few very heat-stable lychee allergens has to be considered.

4. Discussion

In this study, the allergenic potency of seven lychee lots representing at least five different cultivars was compared. Among the Thai cultivars, 'Hong Huey' is often used in industrial processing and is thus also found in form of canned fruits in European markets, whereas 'Bai Dum' and 'Kuang Chao' are mainly sold in domestic fresh fruit markets of the cultivation regions. 'Chacapat' has been increasingly applied in industrial production of frozen fruits. It should be noted that the Thai variety 'Hong Huey' and the South African cultivar 'Mauritius' are systematically closely related to each other, since lychee cultivars are often distributed under different names in the various cultivation countries (Menzel & Simpson, 1991; Aradhya, Zee, & Manschardt, 1995). In particular, 'Hong Huey' is a widely grown cultivar, also cultivated in Australia ('Tai So', 'Big Crop'), the United States of America ('Kwai Mi', 'Charley Tong') and Israel ('Mauritius', 'Tai So'). By analogy, 'Bai Dum' is also known as 'Hak Ip' (United States of America, Taiwan) and 'Haak Yip' or 'Black Leaf' (Australia). Although lychees can be found in European markets throughout the year, using the different harvest seasons in the various producer countries, the availability of different lychee cultivars is limited owing to the multiple designation of varieties. In conclusion, the studied fruit batches reflected typical lychee cultivars available to European consumers.

Both, the four varieties originating from Thailand, and the cultivars from other countries ('Mauritius' and 'Cope' from South Africa and one unknown variety from Madagascar) showed a similar spectrum of IgE-binding proteins with comparable allergenic activity. By analogy, comparable investigations made by Wigotzki, Steinhart, and Paschke (2000) regarding hazelnut allergens only revealed slight differences in the IgE-binding protein patterns with respect to different varieties. In contrast, the allergenic potency of apples depended on their variety (Vieths et al., 1993 & Vieths et al., 1994).

In general, allergenic proteins of the most foodstuffs except fruits and vegetables have been previously reported to be stable during processing (Hefle, 1996; Lehrer, Horner, & Reese, 1996). So far, only very few food allergens were investigated in detail with respect to their process stability (Vieths, Jankiewicz, Aulepp, & Haustein, 1997). Recently, thermostable allergens found in various fruit species have increasingly gained technological interest (Dube et al., 2004). Sufficient decrease of the allergenic potency in hypoallergenic peach juice production by technological means was exclusively achieved by final ultrafiltration due to the high thermal stability of peach allergens, resulting in complete loss of the typical appearance of cloudy peach juices (Brenna et al., 2000). The present investigations of canned lychees with pasteurisation up to approximately 20 min at 90 °C ($P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ = 19 min) indicated the stability of IgE-binding proteins with molecular weights of 14, 40, 43, 55, and 94 kDa. Nevertheless, after 10 min of pasteurisation ($P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ = 10 min) a slight decrease of the allergenic activity could be observed by means of EAST-inhibition. After sterilisation only one IgE-binding protein with a molecular weight of 55 kDa could be detected. On the one hand, Fig. 3 shows detection of less allergens after canning. Thus, the slight decrease of the allergenic potency shown by EAST investigations suggested that the disappeared allergens (25, 28, 32 and 35 kDa) should hardly contribute to the allergenic potency of the whole lychee. On the other hand, further

investigations (not published to date) on the frequency of detection of single allergens by certain sera presented these allergens as important lychee allergens in the fresh fruit. Considering this background, it can be stated that these proteins were no more detectable after pasteurisation by immunoblotting, but their epitopes still had to be present in smaller proteins generated by heat degradation without being accessible to determination in this electrophoresis system. Product storage after canning had no influence on the allergenic potency. Owing to their pH below 4.5, sufficient shelf-life of canned lychees is usually achieved by pasteurisation, applying temperatures below 100 °C as for other acid foods, since the risk of growth and toxin production by *Clostridium botulinum* and other pathogenic bacteria is extremely unlikely. At pH levels of 4.0–4.5, canning processes are aimed at controlling the survival and growth of spore-forming organisms such as *Bacillus coagulans*, *Bacillus polymyxa*, *Bacillus macerans* and the butyric anaerobes. For this purpose, pasteurisation equivalent to 5 and 10 min at 93.3 °C was recommended for products at pH 4.0–4.3 and pH 4.3–4.5, respectively, assuming usual microbial contamination of the raw material (Brown, 1991). In the present study, this corresponded to variants V4–V6, where still no softening or inacceptable discolouration was caused by heating.

Comparable allergological investigations with lychee products do not exist. Hsieh, Moos, and Lin (1995) proved the existence of thermolabile apple allergens. After heating apples at 100 °C for 20 min, no IgE-binding protein was detectable by means of immunoblotting. Nevertheless, residual allergenic potency was found after heat treatments in canned apple products. Equally, Schubert, Steinhart, and Paschke (2003) observed a decrease of allergenic potency during processing of potato, namely the production of potato flakes.

Apart from the cross-reactive 35 kDa protein reported by Wellhausen et al. (1996), several lychee allergens were detected in the fresh fruit, sharing the rather low molecular weights between 14 and 35 kDa with typical cross-reactive food allergens such as e.g., profilins (14.3–15 kDa), isoflavone reductase (32 kDa), and cysteine protease (30–34 kDa) (Mills et al., 2003). Apart from the 14 kDa allergen, the IgE-binding capacity of proteins with lower molecular weight were reduced by pasteurisation, consistently with the reported thermal sensitivity of cross-reactive allergens (Vieths et al, 2002; Mills et al., 2003). Additionally, rather heat-stable allergens with molecular weights ranging from 40 to 97 kDa were detected in all fresh fruit lots. A comparable thermostability was described by Mills et al. (2003) concerning the 11S and 7S globulins of the cupin superfamily. Especially, the lychee allergen at 55 kDa has to be mentioned, since it was solely found after excessive sterilisation destroying sensory product quality. This allergen was striking for both the enormous thermal stability and the tremendous allergenic activity of lychee extracts shown in the EAST-inhibition assays of the cans.

Comparing heat sensitivity of fruit softening, pink discolouration and reduction of the overall allergenic potency of lychees, activation energy increased in the same order. As it was previously shown for conventional mango processing into pulp-containing products typical for this fruit species (Dube et al., 2004), usual canning of lychees ($P^{z=8.9\,^{\circ}C}_{T_{ref}=93.3\,^{\circ}C}$ = 5–10 min) does not allow complete elimination of the allergenic potency. Pasteurisation only contributed to reduce the allergenic potency of allergens with molecular weights of 25, 28, 32 und 35 kDa without significant reduction of the overall allergenic potency. Harsher heat treatments were not useful due to sensory damage and the high thermostability of particular lychee allergens, especially that of 55 kDa.

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