Research Article

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Effects of pre-freezing, puree content and pasteurisation regime on colour stability of strawberry nectar made from puree

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Abstract

BACKGROUND: Heat treatment during processing of strawberry products has been proposed to negatively affect colour stability. Moreover, the role of enzymes with respect to colour stability is ambiguous when consulting the existing literature. The aim of the present study was to investigate the impact of various processing parameters (pre-freezing, puree content, pasteurisation temperature and heating time) on the colour stability and anthocyanin monomer and L-ascorbic acid contents of strawberry nectars made from puree. In addition, the effect of different enzyme activities on colour stability during storage of strawberry nectars was investigated.

RESULTS: Pre-freezing of strawberries before processing had a significant positive effect on the colour stability of nectars made from puree. No significant effect on colour stability was found for higher puree contents. Increasing both the pasteurisation temperature and the heating time had a significant positive effect on colour stability. Results showed that colour degradation during storage was mainly due to residual enzyme activities. The shelf-life of strawberry nectar could be extended about fivefold by adding an enzyme inhibitor.

CONCLUSION: The colour stability of strawberry nectar made from fresh puree may be improved to some extent by an appropriate pasteurisation regime. Enzymes play an important role in colour degradation during storage of the nectar. Inactivation of these enzyme activities, however, could not be achieved even after a heat treatment at 90 °C for 60 min.

Keywords: strawberry; nectar; colour; stability; anthocyanins; L-ascorbic acid; heating

INTRODUCTION

Strawberries and strawberry products, especially products containing puree, are very popular in Western Europe, with the assortment available in supermarkets increasing every year. One significant problem of these strawberry products is colour stability, since the typical red colour associated by the consumer with strawberries is not stable. In order to improve the shelf-life of puree-containing products, natural colorants are added to some of them. The addition of colorants, however, is not allowed for nectars.[1]

Previous studies have indicated a wide range of parameters and conditions that may affect the colour stability of strawberry products during processing and storage. Temperature, L-ascorbic acid content and pH value[2] as well as the presence of certain metal ions, light, oxygen and non-enzymatic browning reactions[3–5] have been related to colour stability.

The influence of the activity of oxidoreductases such as polyphenol oxidase (EC 1.14.18.1) and peroxidase (EC 1.11.1.7) on colour stability and degradation is discussed ambiguously.[6–10] Furthermore, different results on the thermostability of these enzymes have been obtained in previous studies.[6–8,11]

In addition to the above-mentioned factors, colour stability is influenced by self-association (condensation of anthocyanins)[12] and co-pigmentation (interactions of anthocyanins with polyphenols),[13] which can lead to the formation of compounds that have a higher stability than the anthocyanin monomers.[3] Co-pigmentation is the result of an exothermic reaction between the pigment and the co-pigment[14] and is influenced by the concentrations and molecular structures of anthocyanins as well as by the pH value and temperature.[15,16] Higher temperatures

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cause dissociation of the anthocyanin–co-pigment complexes and therefore cause colour degradation. Bakowska *et al*.[4] observed a maximal co-pigmentation effect at pH 3.5 and stated that UV irradiation had a stronger degrading effect on the co-pigmentation complex than heating at 80 °C for 1 h.

The impact of heating on the colour and anthocyanins of several fruit products has been studied previously during processing.[17,18] However, no data are available concerning the effect of thermal treatment on colour stability during storage.

In this study the effects of (i) pre-freezing, (ii) puree content, (iii) pasteurisation temperature and (iv) heating time on the colour stability and anthocyanin monomer and L-ascorbic acid contents of strawberry nectar made from puree were determined and (v) the impact of enzyme activities on colour stability during storage was investigated by adding an enzyme inhibitor (*N*-ethyl maleimide) to strawberry nectar after pasteurisation.

EXPERIMENTAL

Strawberries

Fresh strawberries (*Fragaria* × *ananassa*, cultivars Elsanta (designs 1 and 3) and Malling Pandora (design 2)) were harvested in June and July 2006 and 2007 in Rust and Obergrünbach (Austria) at commercial ripeness during the morning. They were either processed immediately or packed within 2 h (5 kg plastic bags) and stored for 3 weeks at −18 °C. Frozen strawberries were partially thawed at 4 °C for 24 h before they were used for nectar production.

Nectar production

Strawberries were milled with a roller crusher (Wottle, Poysdorf, Austria), sieved with a sieving machine (Wiesböck, Wien, Austria) and milled again with a colloid mill (Fryma, Rheinfelden, Switzerland). The puree was mixed with water, citric acid and sugar in order to obtain nectar (20 L of each variant, 400 and 600 g kg^{-1} puree (Malling Pandora, 400 g kg^{-1} puree: soluble solid content 14 °Brix, titratable acidity 7 g L^{-1})). The nectar was degassed in a vacuum tank at −0.6 bar for 15 min, put into 0.2 L white glass bottles using a vacuum filler (Rapf & Co., Maria Enzerdorf, Austria), pasteurised (pasteurisation temperature of 80 or 90 °C as indicated and heating time of 1–60 min) in a tunnel pasteuriser (Balik, Wien, Austria) and then stored at 20 °C in the dark.

Enzyme inhibition

The enzyme inhibitor *N*-ethyl maleimide (Sigma-Aldrich, Taufkirchen, Germany) was added at 10 mmol L^{-1} to the nectar (design 3, 600 g kg^{-1} puree, 10 min of pasteurisation) after the pasteurisation step.[19]

Chemical and physical analyses

Titratable acidity (TA, g L^{-1}), pH value and total soluble solid content (SSC, °Brix) were measured as described previously.[20] The firmness of fresh strawberries (ten strawberries per variant) was measured using a penetrometer (stamp 10, Setop, Durofel, Tarascon, France) and expressed as Durofel %. Values of colour components L^*, a^* and b^* (CIELAB system) of strawberries, purees and nectars containing puree were measured using reflection methods and a Minolta CM 3500d colorimeter (Osaka, Japan; spectrophotometric method, D65, 30 mm, 10°, gloss excluded, colour data software CM-S100w Version 1.4). The colorimeter was calibrated using a standard white reflector plate (No. 16 471 004).

Table 1. Characterisation of different pasteurisation regimes employed in experimental designs to study strawberry nectars through calculation of pasteurisation units (PU)

Design	Puree content (g kg^{-1})	Temperature (°C)	Heating time (min)	PU
1	400	80	1	20
			30	54
		90	1	174
			30	637
	600	80	1	18
			30	52
		90	1	154
			30	568
2	400	80	1	20
			30	57
		90	1	109
			30	399
3	400	90	10	116
			60	643
	600	90	10	110
			60	854

The calculation of C^* (chroma) and h (hue angle) was carried out as described previously.[21] Colour changes were described by means of an 'acceptance factor' ($AF = a \times h^{-1}$).[22] The anthocyanin monomer (AM) content of strawberries, purees and nectars was measured by high-performance liquid chromatography (HPLC) following the method described previously.[23] All samples were stored at −18 °C before analysis. Results are given as mg pelargonidin 3-*O*-glucoside equivalent L^{-1}. The half-life (HL) of anthocyanin monomers was calculated as described previously.[24] L-Ascorbic acid (AA) content was determined following a modified published method.[25]

In order to quantify the heat input during pasteurisation, the temperature of the nectar was measured every minute directly in the bottle (CTF 84 thermometer, Ellab, Roedovre, Denmark). Pasteurisation units (PU) were calculated as described previously[26] (Table 1).

Statistical analyses

Analyses of pH value, SSC, TA, colour and AM and AA contents were run twice, while measurements of firmness (ten strawberries) were done in triplicate. Statistical analyses were carried out using SPSS 12.0 (Statistical Package for the Social Sciences) (SPSS Statistics, München, Germany). Effects of the various factors were calculated and tests of significance carried out according to Kleppmann.[27] The different experiments performed are based on fractional experimental designs, with experiment 1 based on a 2^4, experiment 2 on a 2^2 and experiment 3 on a 2^3 fractional experimental design. The description of individual factor steps is summarised in Table 2.

RESULTS AND DISCUSSION

Strawberries

Strawberries varied considerably in colour component values, AM content and firmness (Table 3) as well as in the pH value, SSC and TA of purees produced from them (Table 4). Fruits of Elsanta 2006

Table 2. Description of factor steps of experimental designs 1–3 used for studying strawberry nectars made from puree

Design	Factor	Parameter	−	+
1	A	Freezing	No	Yes
	B	Puree content	400 g kg^{-1}	600 g kg^{-1}
	C	Pasteurisation temperature	80 °C	90 °C
	D	Heating time	1 min	30 min
2	A	Pasteurisation temperature	80 °C	90 °C
	B	Heating time	1 min	30 min
3	A	Freezing	No	Yes
	B	Puree content	400 g kg^{-1}	600 g kg^{-1}
	C	Heating time	10 min	60 min

(design 1) seemed to be at the highest ripeness stage (highest AF, low firmness and high AM and AA contents). Malling Pandora (design 2) had the highest pH value and AM content but the lowest SSC. Malling Pandora ripens very late. It has firm fruits which are darker than those of Elsanta owing to a higher AM content.

Design 1

Freezing strawberries before processing did not influence the colour during processing (AF before pasteurisation, A: −0.001) (Table 5). Pre-freezing, however, did improve colour stability significantly during subsequent storage of the nectar (AF after 4 weeks, A: 0.149, $P = 0.001$). This enhancement of colour stability by means of a pre-freezing treatment has not previously been described in the literature and could be of interest for improving colour stability and consumer acceptance. The average loss of AM during pasteurisation was approximately 20%, in good agreement with published data.[28] Pre-freezing resulted in decreased AM content before and after pasteurisation (A: −26, $P = 0.05$ and A: −42, $P = 0.001$ respectively), but the HL of AM increased significantly (A: 883, $P = 0.05$). Thus the greater losses of anthocyanins during pasteurisation of nectars made from frozen strawberries were compensated during storage by a higher stability of the anthocyanins.

The average losses of AA during pasteurisation and after storage for 4 weeks were approximately 11 and 52% respectively, considerably smaller than those reported previously.[29,30] A significant negative effect of pre-freezing on AA could only be observed after pasteurisation (A: −12, $P = 0.01$).

As expected, the higher content of puree resulted in a higher AF, which is based on a higher AM content, but only before and after pasteurisation (B: 0.164, $P = 0.01$ and B: 0.119, $P = 0.001$ respectively). No significant effect of higher puree content on AF was observed after 4 weeks of storage. The positive effect of higher puree content on AM content decreased during storage (B: 59, $P = 0.01$, B: 40, $P = 0.001$ and B: 16, $P = 0.001$ before pasteurisation, after pasteurisation and after 4 weeks respectively), as did the effect on AA content (B: 85, $P = 0.001$, B: 83, $P = 0.001$ and B: 29, $P = 0.01$ before pasteurisation, after pasteurisation and after 4 weeks respectively). According to published results, a higher content of anthocyanins should improve stability through self-association,[31] and Giusti and Wrolstad[32] describe a higher colour stability in products with increased anthocyanin concentration. The results of the present study, however, show that a higher AM content due to an increased puree content improves neither anthocyanin stability nor colour stability during storage. Garzón and Wrolstad[30] also reported a decreasing stability of AM at higher concentrations. These results could be confirmed in this study.

The pasteurisation regime significantly affected the colour stability of strawberry nectars made from puree. Both a higher pasteurisation temperature and a longer heating time improved colour stability (AF after 4 weeks, C: 0.048, $P = 0.05$ and D: 0.047, $P = 0.05$), even though AM and AA were negatively affected by an intensified pasteurisation (AM, C: −26, $P = 0.01$; AA, C: −13, $P = 0.001$ and D: −22, $P = 0.001$). This increase in colour stability may be caused by different factors: on the one hand it may be due to increased co-pigmentation[15] and on the other hand to a more effective inactivation of enzyme activities. The two parameters investigated for the pasteurisation regime, temperature and heating time, show different effects. A higher pasteurisation temperature resulted in a significant increase in the HL of AM (C: 807, $P = 0.05$). The resulting AM content did not differ significantly after 4 weeks of storage. AM content is therefore not a good measure for determining the colour quality of strawberries during storage.[33] The prolonged heating time significantly stabilised the AA content (AA after 4 weeks, D: 34, $P = 0.001$) but had no impact on the HL of AM. This stabilising effect of an intensified heat treatment on AA during storage (CD: 17, $P = 0.05$)[34] may be caused by a more pronounced inactivation of ascorbate peroxidase activity. The role of AA in colour degradation is not clear to date. It has been stated that anthocyanin decomposition is accelerated in the presence of

Table 4. Characterisation of strawberry purees used in different experimental designs to study strawberry nectars: pH value, soluble solid content (SSC, °Brix), titratable acidity (TA, g L^{-1}), anthocyanin monomer (AM) content (g L^{-1}) and L-ascorbic acid (AA) content (mg L^{-1})

Design	pH	SSC	TA	AM	AA
1	3.41 ± 0.5	10.0 ± 0.4	10.4 ± 0.2	255 ± 25	473 ± 12
2	3.90 ± 0.5	7.2 ± 0.4	8.9 ± 0.2	330 ± 18	331 ± 15
3	3.60 ± 0.5	11.0 ± 0.4	9.8 ± 0.2	202 ± 22	ND

ND, not determined.

Table 3. Anthocyanin monomer (AM) content (mg L^{-1}), firmness (Durofel %) and colour component values of fresh strawberries used in different experimental designs to prepare purees and nectars

Design	AM	Firmness	L^*	a^*	b^*	C^*	h
1	318 ± 13	36.9 ± 14.3	35.97 ± 4.56	34.97 ± 5.52	19.70 ± 6.04	40.28 ± 7.40	28.72 ± 5.10
2	362 ± 22	40.2 ± 14.1	34.18 ± 3.68	29.13 ± 3.79	17.32 ± 4.92	34.00 ± 5.53	30.18 ± 4.70
3	210 ± 20	ND	38.18 ± 3.31	38.16 ± 2.67	25.07 ± 4.48	45.76 ± 4.14	33.06 ± 0.14

ND, not determined.

Table 5. Effects of freezing (A), puree content (B), pasteurisation temperature (C) and heating time (D) (design 1) on acceptance factor (AF), anthocyanin monomer (AM) content, L-ascorbic acid (AA) content and half-life (HL) of AM

	Before pasteurisation			After pasteurisation			After 4 weeks			
Effect	AF	AM	AA	AF	AM	AA	AF	AM	AA	HL
T	0.904	139	228	0.942	112	203	0.782	59	97	1073
A	−0.001	**−26***	−16	0	**−42*****	**−12****	**0.149*****	0	−15	**883***
B	**0.164****	**59****	**85*****	**0.119*****	**40*****	**83*****	0.021	**16*****	**29****	−337
AB	0.015	−17	−3	−0.021	−15	**6***	−0.017	−7	−6	−402
C				0.021	**−26****	**−13*****	**0.048***	3	9	**807***
AC				0.013	−3	−1	−0.01	0	−5	610
BC				−0.01	−9	0	0.029	5	6	−192
ABC				−0.008	8	1	−0.013	−4	−4	−398
D				0.01	−14	**−22*****	**0.047***	−2	**34*****	−47
AD				−0.002	5	−2	−0.022	−4	−2	−254
BD				0.003	3	0	0.013	4	9	124
ABD				0.001	−3	3	−0.005	−3	−4	42
CD				0.001	−8	0	0.027	−2	**17***	−134
ACD				0.012	11	−1	−0.02	−2	−3	−315
BCD				0.009	−1	1	0.015	1	7	173
ABCD				0.007	2	0	−0.012	−1	−3	65

[a] T, average; A, main effect of A, etc.; AB, interaction of A and B, etc.
* $P = 0.05$; ** $P = 0.01$; *** $P = 0.001$.

Table 6. Effects of pasteurisation temperature (A) and heating time (B) (design 2) on acceptance factor (AF), anthocyanin monomer (AM) content and L-ascorbic acid (AA) content of strawberry nectars

	After pasteurisation			After 4 weeks	
Effect	AF	AM	AA	AF	AA
T	0.843	138	99	0.678	37
A	0.007	0	−15	0.012	5
B	−0.007	**−11****	**−28***	0.002	**17***
AB	−0.011	**−23*****	2	−0.001	4

* $P = 0.05$; ** $P = 0.01$; *** $P = 0.001$.

Table 7. Effects of freezing (A), puree content (B) and heating time (C) (design 3) on acceptance factor (AF) and anthocyanin monomer (AM) content of strawberry nectars made from puree

	After pasteurisation		After 4 weeks		After 6 weeks
Effect	AF	AM	AF	AM	AF
T	0.95	70	0.708	24	0.586
A	0.027	−2	0.093	−1	**0.145***
B	**0.089****	**21***	0.032	**5***	0.024
AB	−0.008	7	−0.028	1	−0.051
C	**−0.077****	**−28****	−0.003	**−8****	0.029
AC	−0.017	2	0.06	1	0.066
BC	−0.024	−6	0.013	1	0.032
ABC	0.006	−4	−0.007	−1	−0.008

* $P = 0.05$; ** $P = 0.01$.

AA,[35] and the formation of hydrogen peroxide resulting from AA oxidation may influence anthocyanin stability. Anthocyanins, however, are also thought to be protected by AA against enzymatic degradation.[36]

We propose that colour stability is influenced by the intense heat treatment in two ways, i.e. enhanced formation of co-pigments and increased inactivation of enzyme activities. The highest heat treatment applied in our study (90 °C, 30 min), however, could not stabilise colour more than the pre-freezing treatment.

Design 2

In design 2 the chosen processing parameters (pasteurisation temperature and heating time) had no significant effects on colour both directly after pasteurisation and after 4 weeks of storage (Table 6). Even after 6 and 8 weeks of storage, no significant effects on AF were observed. Pre-freezing had the biggest effect on AF after 4 weeks of storage in design 1. The other parameters selected for that design (temperature and heating time) had a significantly lower impact on colour stability. As only frozen strawberries were processed in the experiment of design 2, this may be one reason for the lack of a significant influence of the chosen parameters on colour stability in experimental design 2. Another reason may be the use of a different variety of strawberries for this design (Tables 3 and 4). An intensified heat treatment led to significant losses of anthocyanins (B: −11, $P = 0.01$ and AB: −23, $P = 0.001$) (Table 6). Furthermore, a prolonged heating time negatively affected the AA content after pasteurisation (B: −28, $P = 0.05$). After 4 weeks of storage an increase in AA stability could be determined (B: 17, $P = 0.05$). This effect was noticed in design 1 as well.

Design 3

In design 3 the factors freezing (A), puree content (B) and heating time (C; with further increased values of 10 and 60 min as compared with designs 1 and 2) were studied with respect to colour stability and AM (Table 7). Freezing had no significant effect on AF after

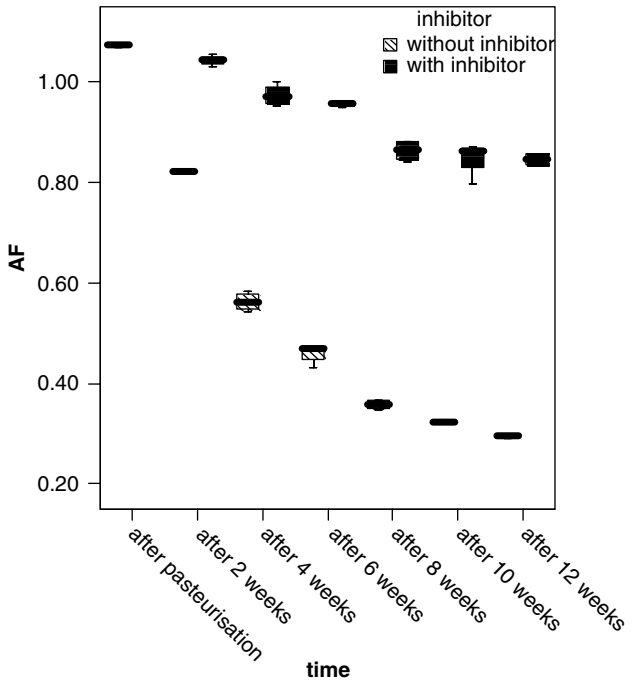

Figure 1. Effect of enzyme inhibitor (*N*-ethyl maleimide) on colour degradation (acceptance factor, AF) during storage of strawberry nectars prepared from puree (design 3).

pasteurisation and after 4 weeks of storage, but a positive effect was observed after 6 weeks of storage (A: 0.145, $P = 0.05$). This advantageous effect on AF increased continuously during prolonged storage (e.g. after 8 weeks, A: 0.151, $P = 0.05$). Puree content positively influenced AF after pasteurisation (B: 0.089, $P = 0.01$) and AM content after pasteurisation and after 4 weeks of storage (B: 21, $P = 0.05$ and B: 5, $P = 0.05$ respectively). As in design 1, no increase in colour stability, which might be caused by higher AM content (higher puree content), was measured. The positive effect of higher puree content on AF observed directly after pasteurisation decreased during storage. Extension of the heating time from 10 to 60 min degraded colour during pasteurisation (AF after pasteurisation, C: -0.077, $P = 0,01$) as well as AM content after pasteurisation and after 4 weeks of storage (C: -28, $P = 0.01$ and C: -8, $P = 0.01$ respectively). However, the colour of nectars heated for longer times was more stable during storage. This effect was noticed in design 1 as well.

No significant effect of the chosen parameters of design 3 was found for the HL, with the average HL of design 3 calculated as 436 ± 35 h. Further, addition of *N*-ethyl maleimide did not affect the HL (407 h). In contrast, colour stability increased dramatically on addition of this enzyme inhibitor (Fig. 1). The main differences in aging between nectars with and without inhibitor addition are found in b^* and h. While b^* typically decreases only to a small extent and h increases in the absence of inhibitor, b^* decreased considerably more and h decreased as well in nectars with added inhibitor. This indicates that the colour did not change to orange but to violet and blue in the presence of the enzyme inhibitor. This effect can be attributed to the formation of co-pigments (bathochromic shift).[37]

Compared with nectars without inhibitor addition, colour degradation (i.e. decrease in AF) in nectars containing the inhibitor was reduced by about 80% during the first 4 weeks of storage (measured data: -0.515 AF (-48%) and -0.103 AF (-9.6%) for nectars without and with addition of inhibitor respectively). This indicates that the shelf-life of nectars made from strawberry puree could be extended up to fivefold if enzymes are inactivated during processing.

This result confirms the importance of endogenous enzyme activities in colour degradation during storage of strawberry nectars made from puree.[38] Based on previously published results, we did not expect enzyme activities to affect colour stability to such a large extent. Spayd *et al.*[9] did not report any influence of polyphenol oxidase (PPO) and peroxidase (POD) activities on the colour of strawberry puree. Previous studies showed that, for strawberries, POD is more thermolabile than PPO,[7] with PPO from Elsanta losing 95% of its activity during a 60 min incubation at 60 °C and POD losing 60–80% of its activity during a 60 min incubation at 50 °C. Serradell *et al.*[8] reported an almost complete loss of PPO activity even after 30 min of heating at 65 °C. Based on these data, the times required to halve the activities of PPO and POD from Elsanta at 80 °C were calculated to be 1.7 and 1.4 min respectively.[7] These values would indicate that both PPO and POD activities are completely lost after the chosen heat treatments used in the experimental design of our study. Yet, this is contradicted by the clear and significant effect of the enzyme inhibitor *N*-ethyl maleimide that was added after pasteurisation. Recently, a thermostable peroxidase isoenzyme was detected in strawberries.[11] After heat treatment of drained canned strawberries, 6.4–26.2% of residual POD activity was determined. Gössinger *et al.*[39] also found residual activities of PPO and POD in strawberry nectars after pasteurisation. These contradictory data clearly warrant a more detailed investigation on different oxidoreductase activities and isoenzymes found in strawberries.

CONCLUSIONS

The results of the statistically designed experiments in our study show that the colour stability of nectars made from fresh puree may be increased to some extent by an appropriate pasteurisation regime. Whereas a higher heating temperature affected the half-life of anthocyanins, an extension of the heating time improved the stability of L-ascorbic acid. We propose that an intensified pasteurisation process results in increased inactivation of detrimental enzyme activities such as peroxidase. Because of these lower enzyme activities, co-pigmentation can proceed over an extended period of time during storage. In addition, a higher stability of L-ascorbic acid supports colour stability. The importance of enzyme activities in colour degradation during storage could be demonstrated by the addition of a chemical inhibitor, *N*-ethyl maleimide. Based on the results obtained in our study, more than 80% of the colour degradation in nectars from puree could be the result of detrimental enzyme activities. The inactivation of these enzymes, however, could not be achieved even after a heat treatment for 60 min at 90 °C. Further investigations are necessary to obtain more detailed information about enzyme inactivation during processing of strawberry products.

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