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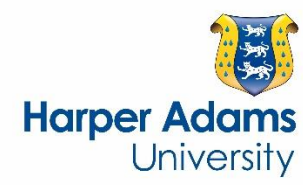

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Dewatering treatments to increase dry matter content of the brown seaweed, kelp (*Laminaria digitata* ((Hudson) JV Lamouroux))

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Abstract

Macroalgal water content is an on-going problem for the use of readily accessible seaweeds in sustainable biorefining, including fuel production. Silage is a reduced-water, compactable, easily stored, transportable material. Ensiling could establish a non-seasonal supply of preserved algal biomass, but requires high initial dry matter content to mitigate environmental pollution risks from effluent. This study investigated potential dewatering methods for kelp harvested throughout the year. Treatments included air-drying, osmotic media and acids. Significant interactions between treatment and harvest-time were observed for traits of interest. Fresh weight loss during treatment was composed of changes in water and dry matter content. Air-drying gave reliable increase in final dry matter content; in summer and autumn 30% dry matter content was reached after 24 h. Dilute hydrochloric acid reduced stickiness and rendered material suitable for dewatering by screw-pressing; it may be possible to use the consequent pH reduction to promote efficient preservation.

Keywords: biorefining-feedstock preservation; ensiling; macroalgae; seasonal variation; silage effluent production/reduction.

Abbreviations: DM, dry matter

1. Introduction

Kelps, seaweeds within the Phaeophyceae, have high photochemical efficiencies and growth rates in temperate coastal regions. Consequently these macroalgal species offer the opportunity to produce large quantities of environmentally and socially advantageous 'green' biomass for biorenewable applications by avoiding conflict with crop production for the use of agricultural land and fresh water. The macroalgal industry has grown dramatically over recent years in Asia. Over 23 million dry tons macroalgae were produced by aquaculture in 2012, mostly for human consumption (Loureiro et al., 2015). Globally there is scope for further expansion of this production for an increasing range of markets. Opportunities for biotechnology and sustainable fuel production have been recognised, but have not currently been exploited to their full potential (Milledge et al., 2014; Loureiro et al., 2015; Suutari et al., 2015). Further research is required on both cultural aspects (e.g. the development of genetic resources, crop cultivation in temperate zones, disease and pest management, providing a year-round supply of biomass) and bioprocessing technologies and associated challenges (e.g. biomass preservation, storage, dewatering, transport). Dewatering is of particular interest for sustainable fuel applications because wet biomass is subject to rapid deterioration in quality, is heavy to transport, bulky to store and the fossil energy (and economic) costs of traditional drying processes can be high (Milledge et al., 2014; Herrmann et al., 2015; Milledge and Harvey, 2016a; Soomro et al., 2016).

Macroalgae, like microalgae and most green plant material, have high water content (Adams et al., 2011; Suutari et al., 2015). Screw-pressing has been shown to be an effective initial treatment for reducing the water content of forage crops such as grasses and alfalfa for biorefining purposes (Winters et al., 2010; Takara and Khanal, 2011; Kamm et al., 2016). The resulting juice is rich in organic and inorganic

compounds and can be channelled into other applications for the production of high-value-added products. However, screw-pressing has proved unsuitable for dewatering fresh brown seaweeds in initial trials because the material comes through the press as a sticky mass without producing any juice (unpublished observations). Other approaches to dewatering and/or preserving macroalgal feedstocks are required.

Recent studies have investigated the potential of ensiling methodology to preserve seaweed for anaerobic digestion (Herrmann et al., 2015; Milledge and Harvey, 2016b), building on work first published over sixty years ago by Black (1955). Silage is created from fresh biomass through rapid acidification to about pH 4 or below (Johnson et al., 2004) under anaerobic conditions in a silo or bale. It is produced either chemically by adding organic acid or naturally through fermentation by lactic acid-producing bacteria and yields a stable moisture-containing material preserved in an acidic medium. Traditionally this has provided feed for livestock when fresh forage is limited or unavailable, and in Western Europe silage is now the major form of conserved ruminant feed. Similarly ensiling could establish a non-seasonal supply of material for biorenewable applications. Reported acidification rates are variable, but both Herrmann et al. (2015) and Milledge and Harvey (2016b) concluded that ensiling was an effective, low energy-loss method of preserving algal material.

The ensiling of forage crops, particularly in bunker silos, potentially creates effluent as water leaches from the biomass, with associated risks of contamination to the soil and water courses. Effluent production is commonly controlled when ensiling terrestrial material through increasing dry matter (DM) content to around 30% by wilting cut crops in the field prior to ensiling (Haigh, 1994; Wright et al., 2000). As a result of wilting or water loss in effluent, ensiled livestock feed generally has around 15% lower water content than fresh forage (Wright et al., 2000). Effluent production

was high during macroalgal ensiling trials (Herrmann et al., 2015; Milledge and Harvey, 2016a, 2016b). While effluent may contain useful compounds and could be viewed as a resource, its production is uncontrolled and it is also a potential pollutant. It would be preferable to remove water in a more controlled manner before ensiling in an analogous approach to that used in the production of grass silage. Macroalgal water content is typically 74 – 89% (Herrmann et al., 2015). However reductions of only 1-2% water content between fresh and ensiled biomass have been reported for macroalgae (Herrmann et al., 2015; Milledge and Harvey, 2016b). Decreased water content improves costs and reduces energy consumption for drying where processes require dried and / or pelleted feedstock (Milledge et al., 2015). Ensiled seaweed feedstock that can be readily compacted and easily stored and transported is viewed as an economic proposition as a contributor to sustainable transport fuels such as drop-in HGV diesel and aviation kerosene (*via* gasification and Fischer-Tropsch technologies) (Milledge and Harvey, 2016a).

Herrmann et al. (2015) and Milledge and Harvey (2016b) did not consider any pre-treatments to reduce pre-ensiling water content in macroalgae. A number of types of pre-ensiling treatment are potentially of value. These include treatments that in themselves reduce water content, treatments which make the macroalgal material amenable to screw-pressing for additional dewatering, and treatments to otherwise favour good ensiling by acting like silage additives. The simplest treatment that would be expected to increase DM content is air drying, the equivalent of wilting forage in the field. Media with a higher osmotic potential than that of the macroalgal material will draw water out by osmosis. Acid treatments will reduce initial pH which would be expected to lead to good algal preservation enhancing the early stages of ensiling (Adams et al., 2009; Barbot et al., 2015). Additionally acids may alter the physical (eg

cell wall porosity) and/or biochemical nature of the biomass with consequent effects on subsequent dewatering processes like screw-pressing. Acids which hydrolyse alginates would be expected to reduce stickiness and the consequent release of small solute molecules may also affect osmotic balance.

In this study a range of treatments including drying, application of media with osmotic properties and both organic and mineral acids have been applied to wild-collected *Laminaria digitata* ((Hudson) JV Lamouroux) to study their effects on dewatering. Subsequently, treated material was tested for juice production in a screw-press to investigate whether further dewatering and low-cost processing was possible. Macroalgae were collected at different times of year to examine the influence of seasonal variation in tissue composition (Adams et al., 2011; Schmid et al., 2014) on the effects of the dewatering treatments.

2. Materials and Methods

2.1. Macroalgal material.

Kelp, *L digitata*, was sourced from wild stock on rocky outcrops at Aberystwyth north beach (ordinance survey reference SN 582824) during afternoon spring low tides in April, July, October and January. Material for the time-course experiment was harvested in August, and for the neutralisation experiment in October. Three to four kilograms blade material was cut from the stipe on each occasion and was returned to the laboratory within 1 h. The seaweed was stored in sealed buckets at 4 ^{o}C overnight. Samples of seawater were taken at the same time from beside the collection point and also stored at 4^{o}C. Initial macroalgal DM content (%) was determined by oven drying at 70 ^{o}C for 6-7 days.

2.2. Dewatering treatments.

All reagents for the treatments (Table 1) were analytical grade and are widely available, except for Crimpstore silage additive (Crimpstore 2000S; Kemira Oyj, Finland) which is a mixture of formic, propionic and benzoic acids plus ammonium formate with a trace of formamide as a preservative. Air drying took place on the laboratory bench at ambient temperature and humidity with daytime irradiance from standard laboratory artificial lighting. Blade material (approximately 50 g) without epizoans was selected, blotted dry, weighed and placed with the treatment in 1 L lidded beakers at room temperature. Treatments were set up in random order within three replicate blocks on the laboratory bench. After 24 h the treated macroalgal material was removed from the beaker, briefly rinsed in water, blotted dry, reweighed and then sealed into grip-seal plastic bags. These bags were stored at 4 ^{o}C until the samples were screw-pressed.

Screw-pressing (Green Star Vegetable Juicer GS-1000; Tribest Corporation) was completed within the minimum possible time and always within 24 h after the treatments finished. The volume of juice produced and the fresh and dry (after 6-7 days at 70 ^{o}C as in 2.1 above) weights of the solid residue were recorded. Screw-pressing samples of this size led to significant non-recovery of up to 40% (average 14%) biomass which remained as un-pressed material in the screw of the juicer. Recovery of biomass from the press was estimated from the weight of input material and the quantified outputs, namely the combined fresh weight of solid residue and juice (using a density of 1.1 g/ml based on several random measurements on juice samples). Such losses as a consequence of scale at this stage limit the value of calculating whole-process biomass recovery.

Five traits which summarise the effects of potential dewatering treatments and of screw-pressing on macroalgal material were derived and the results are expressed per 50

g sample. The overall change in (fresh) weight was obtained from the initial and post-treatment (24 h) fresh weights. Changes in fresh weight caused by the dewatering treatment could result from changes in water content and /or in the solute component of DM. Therefore dry weight post-treatment at 24 h was calculated from the screw-press residue dry weight plus 9.1% weight of juice, both corrected for the losses in the press assuming equal percentage losses of solid residue and juice. Water content was calculated from the post-treatment fresh and dry weights. The juice produced by screw-pressing was similarly corrected for losses in the press. Final DM content (%) was obtained from the fresh and dry weights of the pressed residue.

2.3. Time-course experiment.

Dewatering treatments following the procedures described in 2.2 above were repeated with three concentrations of hydrochloric acid (0.05M, 0. 1M and 0. 2M) plus air-drying and saline controls, with samples taken and tested at time points from 0 - 48h.

2.4. Neutralisation experiment

Dewatering treatments following the procedures described in 2.2 above were repeated with 0.1M hydrochloric acid for 16 h overnight. The treatment solution was neutralised with excess powdered $CaCO_3$ and agitated at 15 – 30 min intervals. Samples were taken and tested after 1 min, 30 min and 4 h and compared with un-neutralised controls. The pH of treatment solutions and screw-press juices was monitored with indicator strips (BDH 315342Q, pH 2-9; VWR international)

2.5. Statistical analysis

Data were analysed using the standard menu-driven procedures within GenStat edition 13 (VSN International). The treatment replicates were used as blocs to account for temporal variation from sample processing time in two-way analysis of variance (ANOVA). *Post-hoc* multiple comparison analysis was carried out with the Tukey test

using 95% confidence limits. Correlations were calculated as the product moment correlation coefficient for pair-wise combinations.

3. Results and Discussion

3.1 Dewatering treatments

The fourteen potential dewatering treatments tested in this study (Table 1) included examples based on drying, osmotic effects and acids, and were chosen to be compatible with biomass processing and ensiling protocols. Air drying is a low tech, low energy, low cost procedure which could be applied under a range of circumstances. Sodium chloride was the basis of the osmotic media; seawater, a saline solution expected to be hypertonic compared with the natural environment of the algal material and dry salt crystals were contrasted with hypotonic pure water. Organic acids which are, or have been, used as commercial silage additives and which could be applied as small volumes of concentrated liquids spread evenly over the algal surface in a manner comparable with field ensiling methods were chosen. Ammonium formate was included as a ‘dry’ treatment as it can be the additive of choice on-farm because it is less corrosive to agricultural machinery. Two mineral acids with a stronger acid effect but which could be applied as small volumes of concentrated liquids were also tested. Additionally, the mineral acids together with formic acid were applied as treatment solutions for comparison with application of concentrated acid. Sulphuric acid was considered, but would be too corrosive to apply to biological material in the concentrated state.

3.2 Effects of dewatering treatments on kelp

The effects of potential dewatering treatments on samples of kelp treated for 24 h are shown in Figure 1. There were significant effects of treatment ($P<0.001$) in two-

way ANOVA for all five traits of interest. Sea water provided a good control treatment producing little change in fresh weight after 24 h (Figure 1A). Loss of fresh weight was observed in all other treated kelp samples with the exception of ultrapure water, where osmotic uptake of the water caused a substantial increase in weight and concurrent change in water content. The greatest loss of fresh weight occurred with air drying. The data for water content were similar to those for fresh weight indicating that water loss constituted the major part of the weight change (Figure 1B). However, biomass weight loss was not solely due to water content as illustrated by the effects of mineral acid treatment on fresh weight and water content (Figure 1A and 1B). Loss of DM as dissolved solutes also occurred leading to decreases in sample dry weight (Figure 1C). There is no biological mechanism for a detectable increase in dry weight over the timescale and conditions of the dewatering treatments, so the apparent increase in dry weight of material from the dry salt treatment which occurred in all replicates (Figure 1C) has to be an artefact. The additional weight can only have come from the treatment medium, which in this case was pure salt. It is hypothesised that salt became strongly adsorbed (or absorbed) to the kelp during treatment leading to incomplete removal when the samples were rinsed. This increased dry weight led to high %DM in the dry-salted samples; these data are therefore also anomalous and should be disregarded. Acids applied as 1% solutions resulted in significantly greater loss of sample dry weight than any other treatments except ultrapure water (Figure 1C). Dry weight loss from seaweed during treatments applied as solutions may to some degree be non-specific (Adams et al., 2015). These authors have shown that 'washing' macroalgal material with water during sample preparation for fermentation and anaerobic digestion experiments reduced water-soluble carbohydrate content. It is possible that other highly water-soluble metabolites are similarly affected. However, washing does remove most salt

deriving from sea water and Adams et al. (2015) showed it was of benefit where saline conditions may compromise later processing steps.

Only the mineral acids were effective in producing material that would yield juice in a screw-press (Figure 1D), probably because they were the treatments which removed the stickiness caused by alginates. 1% hydrochloric acid solution produced the most juice, and concentrated phosphoric acid treatment the least. The final %DM of macroalgal material was increased from initial %DM in most treatments (Figure 1E). Only material from the ultrapure water treatment had a significantly lower %DM after 24 h treatment resulting from uptake of water during that time. Disregarding dry salting which as discussed above is thought to have produced an artificially high %DM, air drying and treatment with concentrated mineral acid resulted in kelp material with around 25% DM, significantly higher than any of the other treatments.

3.3 Seasonal variation

The effect of collecting material at different times of year is shown on Figure 2. There were significant effects of harvest date ($P<0.001$) in two-way ANOVA for all five traits of interest in this study. Loss of fresh weight and water content were greatest for kelp samples obtained in January and April and least for those collected in July (Figure 2A and 2B). Loss of biomass dry weight was greatest in July and lowest in April with all months being significantly different from each other (Figure 2C). More juice could be pressed from the treated kelp material in January than in the other months (Figure 2D). DM content data are shown on Figure 2E. The initial %DM content of the kelp used in the study was highest in July and October. After dewatering treatment final biomass %DM was highest in October and lowest in April, and all months were significantly different from each other. Seasonal variation in %DM may result from

differences in soluble carbohydrate content over the year as previously demonstrated by other researchers (Adams et al., 2011; Schiener et al., 2015).

3.4 Variation in treatment effects with harvest date

The effects of different dewatering treatments on changes in fresh weight and water content were the same at all times of year examined. However, there was a significant interaction ($P<0.001$) between dewatering treatment and month for the change in biomass dry weight, the juice produced by screw-pressing and final DM content. The statistical analysis was carried out with the full data set, but for clarity only interaction level means for selected traits with notable effects are shown on Figure 3. The greatest loss of dry weight occurred for samples of kelp treated with mineral acid solutions after harvest in July and October (Figure 3A). Juice was only produced by screw-pressing ultrapure water treated kelp samples which were collected in July and October (Figure 3B). Macroalgae harvested in January and treated with1% hydrochloric acid solution gave a particularly high juice yield. This material also showed highest increase in %DM from T0 (Figure 3C) and a final DM content of over 30%, the highest in the study. In contrast decreases in kelp %DM following hydrochloric acid solution treatment and screw-pressing were observed for samples harvested in July and October.

3.5 Relationships between traits

July and October, the months when initial %DM content was highest, were also when the greatest dry weight losses occurred with some dewatering treatments. Therefore, the relationship between initial %DM content and treatment effects on selected traits was investigated further with correlation analysis. As decreases in weight have been represented with a negative sign in this study, the largest effects are, consequently, numerically the smallest numbers. To avoid confounding the direction of the correlations, the magnitude of the change in weight was analysed disregarding the

direction sign. Across all treatments and dates initial %DM was highly correlated with final %DM ($P<0.001$) and change in dry weight ($P<0.001$) (Table 2A). The correlation coefficients for the individual treatments are shown on Table 2B. There were significant correlations between initial and final %DM for many treatments. However the significant correlations with initial %DM for mineral acid treatments were for the change in the dry weight of the macroalgal material. Material with a higher initial %DM content lost more solutes to the treatment solution.

3.6 Time-scale of treatment effects

The relationship between acid concentration and treatment contact time was investigated as the data in 3.2 – 3.5 above provide no information on whether maximum effects were observed. There were highly significant effects of treatment, time and their interaction (Table 3). The samples in saline solution included as a control unexpectedly took up water during the experiment indicating the treatment medium must have been hypotonic to the material, perhaps because of high solute content in late summer. Increasing acid concentration increased fresh weight loss through effects on both water and DM loss, but there were only significant differences (Tukey) between the acid treatments for juice production during screw-pressing. With the exception of the kelp samples subjected to air drying, which steadily lost water and fresh weight over time with little change in dry weight, the changes were not linear (Figure 4). The acid treatments caused an initial rapid loss of weight which levelled off after 6 – 12 hours, with little further change after 24 hours. Higher concentrations of acid had a rapid initial effect, but this levelled off earlier rather than having a greater total effect. Treatment for 12-24 hours produced maximum effects with 0.1 and 0.2M acid, but 0.05M required around 36 hours. Juice production was highest after 48 hours in 0.2M acid (Figure 4D) and it took longer for material in 0.05M acid to become amenable to screw-pressing.

After 6 hours there was little further change in final DM content of material subjected to the different acid treatments (Figure 4E).

3.7 Considerations for processing

The results of this study provide data to inform optimisation of processing protocols for kelp ensilage and storage. In view of the variation of treatment effects with sampling date, the season of harvest may influence the most useful approaches. Air drying was the one treatment to reliably and consistently increase DM content on all harvest dates. Natural air drying is a low technology, low energy, low input method and has the advantage that no DM is leached from the macroalgal material. Maximum energy content is therefore retained and is available for subsequent processing through to sustainable fuels. Increases of 7.2 and 7.5 %DM were observed in July and October respectively, which together with the initial high DM content took final %DM to 29 and 30%. Although the optimum DM content for macroalgae is unknown any loss of water content will be beneficial for ensiling. A DM content of around 30% is considered optimal for minimising effluent and producing good quality silage with forage grasses (Haigh, 1994), and so these water losses may be satisfactory for good algal preservation. Decreases in kelp final %DM were observed for samples harvested in July and October following 1% hydrochloric acid solution treatment and screw-pressing. Even with high initial %DM content, the final %DM content was around only 15%. Although it may be possible to recover useful compounds from treatment media and juices when solute loss is high, air drying may be the most appropriate method for pre-treating kelps harvested during summer and early autumn.

Hydrochloric acid treatment was the most promising treatment for conditioning material for screw-pressing. Application by immersion in dilute solutions led to the greatest juice production, but did not always lead to increases in DM content due to loss

of the dissolved solute component of DM during treatment. However, this method is easy to standardise and apply consistently. Furthermore it was the most effective at increasing final DM content in January, with an increase of 14.7% DM leading to a final %DM of 31%. It may, therefore, be the preferred technology for kelp harvested in winter as air drying will less effective at lower ambient temperatures. Even in the laboratory environment lower (night) temperatures led to a final DM content of only 22% after air drying. It may be possible to recover useful, potentially high value, compounds from the treatment media and juice to maximise the economic potential of the algal biomass. The effects of hydrochloric acid solutions on kelp observed in the seasonal experiments will have occurred within 12h (1% is approximately 0.12M) and so could be achieved overnight. As the same effects can be obtained with higher concentrations of acid for short periods of time or more dilute acid for longer periods, there are trade-offs to be considered between treatment-time and the amount of acid used when designing processing protocols, because the acid used must be disposed of or recycled for further use.

Acid preparations, like Crimpstore, are routinely used as silage additives to aid preservation and improve quality, but neither this product nor the organic acids tested were effective pre-ensiling dewatering treatments for kelp. Stronger mineral acids were required to hydrolyse alginates and remove stickiness for effective screw-pressing. However 1% hydrochloric acid has a pH just under 2. The juice from treated material also had pH 2; hydrochloric acid treatment negated the relatively high buffering capacity demonstrated by Herrmann et al. (2015). A pH of 4 or under is considered to indicate good preservation for grass silage (Johnson et al., 2004), but pH 2 may not be desirable for the labour force, processing machinery or for biomass preservation. Neutralisation between dewatering treatment and screw-pressing significantly reduced

the acidity of the juice without affecting any of the traits of interest. The pH change was time dependent (Figure 5); an immediate rise in pH to around 3.5 was followed by a steady increase to near treatment-medium pH over the following 4 h. This may represent slow release of acid from the algal biomass as regular agitation to resuspend solid $CaCO_3$ was required to maintain the treatment solution at pH 5.5. Neutralisation times to match the desired biomass and juice pH values can therefore be chosen during processing.

Mean dry biomass recovery (not including solutes in treatment solutions and juices which are potentially recoverable) from these pre-ensiling processes was 70%. Recoveries ranged from 43% for hydrochloric acid solution, which lost most solutes and produced most juice, to 87% for sea water. These estimates are strongly biased for most treatments by losses in the order of 14% in the screw-press at laboratory scale. Recovery from pressing would be higher on scale-up. Losses from solute leaching in the initial dewatering pre-treatments can be seen on Figure 1C in the changes in dry weight for a standard 50g sample.

4. Conclusions

Pre-ensiling treatments which significantly increased macroalgal DM content have been identified, although it is not known if optimum values for ensiling were reached. Air drying increased %DM with minimal loss of DM and maintenance of maximum energy content, which is beneficial for sustainable fuel production. Treatment with hydrochloric acid successfully conditioned material for screw-pressing. Immersion led to greatest juice production, but not always to significant increases in %DM. However, immersion methods are easy to standardise and it may be possible to recover

high-value compounds from treatment solutions and juices. The preferred ensiling pre-treatment may depend on the date of harvest.

Supplementary Material

Supplementary Figure S1 is a version of Figure 3 containing the full dataset

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Figure Captions

Figure 1. Effects of the application of different dewatering treatments to kelp for 24 h. A. Change in fresh weight (g/50g material) from time zero (T0). B. Change in water content (g/50g material) from T0. C. Change in dry weight (g/50g material) from T0. D. Juice produced by screw-pressing after treatment (ml/50g material). E. Final dry matter content (%) following dewatering treatment and screw-pressing. Mean initial %DM at T0 is indicated by the horizontal line. Data are treatment means (n=12). Abbreviations for the different treatments are as shown in Table 1. The least significant difference at the 5% level is indicated. Bars marked by the same letter are not significantly different at the 5% level as analysed by the Tukey multiple comparison test.

Figure 2. Seasonal variation in effects on macroalgal traits. A. Change in fresh weight (g/50g material) from T0. B. Change in water content (g/50g material) from T0. C. Change in dry weight (g/50g material) from T0. D. Juice produced by screw-pressing after treatment (ml/50g material). E. Final dry matter content (%) following dewatering treatment and screw-pressing. Initial %DM at T0 for each month is indicated by the horizontal lines. Data are seasonal means (n = 42). The least significant difference at the 5% level is indicated. Bars marked by the same letter are not significantly different at the 5% level as analysed by the Tukey multiple comparison test.

Figure 3. Interaction between selected dewatering treatments and time of year for three traits. A. Change in dry weight (g/50g material) from T0. B. Final dry matter content (%) following dewatering treatment and screw-pressing. Mean initial %DM at T0 for the four times of year is indicated by the vertical black lines across each data set. C. Juice produced by screw-pressing after treatment (ml/50g material). The treatments are

shown as air drying, open bars; sea water, solid grey bars; ultrapure water, solid black bars; concentrated hydrochloric acid, hatched (sloped) bars; hydrochloric acid solution, hatched (square) bars; concentrated phosphoric acid, cross hatched (sloped) bars; phosphoric acid solution, cross hatched (square) bars. Data are all interaction means (n = 3). Statistical effects are from analysis of the full data set with all treatments included. The least significant difference at the 5% level is indicated. Bars marked by the same letter are not significantly different at the 5% level as analysed by the Tukey multiple comparison test.

Figure 4. Time-scale of treatment effects on kelp over a 48 hour period. A. Fresh weight (g/50g sample), B. Water content (ml/50g sample), C. Dry weight (g/50g sample), D. Juice production in a screw-press (ml/50g sample) and E. Final DM content (%). 0.05M hydrochloric acid, open squares; 0.1M hydrochloric acid, grey squares; 0.2M hydrochloric acid, black squares; air drying, open circles; saline, open triangles. Data are interaction means (n = 3) and the 5%LSD is indicated.

Figure 5. Effect of neutralising the hydrochloric acid treatment medium on the pH of juice produced during screw-pressing. Treatment media, open symbols with dashed lines; juice, closed symbols with continuous lines; squares, acid media; circles, with neutralisation at time zero. Data are interaction means (n = 3) and the 5%LSD is indicated. Juice pH data points marked by the same letter are not significantly different at the 5% level as analysed by the Tukey multiple comparison test.

Table 1. Treatments applied to 50g seaweed in 1 L lidded beakers for 24 h at room temperature.

CODE	TREATMENT	APPLIED AS	
AIR	Air drying		Loosely folded, no lid
SALT	Dry salting (NaCl)	10g	Shaken evenly over alga
FORMATE	Dry ammonium formate crystals	5g	Shaken evenly over alga
SEA	Sea water	450ml	Alga immersed
SALINE	Saline solution (10%)	450ml	Alga immersed
DI	Ultrapure water	450ml	Alga immersed
FORM C	Concentrated formic acid	2ml	Evenly over algal surface
FORM S	Formic acid solution (1%)	450ml	Alga immersed
PROP C	Concentrated propionic acid	2ml	Evenly over algal surface
CRIMP C	Concentrated Crimpstore silage additive	2ml	Evenly over algal surface
HCl C	Concentrated hydrochloric acid	2ml	Evenly over algal surface
HCl S	Hydrochloric acid solution (1%)	450ml	Alga immersed
PHOS C	Concentrated phosphoric acid	2ml	Evenly over algal surface
PHOS S	Phosphoric acid solution (1%)	450ml	Alga immersed

Table 2. Correlations between initial %DM content and those traits showing a significant treatment by season interaction. A. Correlation matrix across seasons and treatments, n = 168. B. Correlation coefficients by treatment n = 12. Significant correlations are indicated *P<0.05, **P<0.01, ***P<0.001.

A				
Initial %DM	-			
Change dry wt	0.3031***	-		
Juice	-0.1091	0.3224***	-	
Final %DM	0.3269***	-0.2285**	0.1967*	-
	Initial %DM	Change dry wt	Juice	Final %DM

B			
Treatment	Coefficients for correlation with initial %DM		
	Change dry wt	Juice	Final %DM
AIR	-0.1075	—	0.9370***
SALT	0.3270	—	0.4490
FORMATE	0.1777	—	0.7153**
SEA	0.5993*	—	0.8058**
SALINE	0.3203	—	0.5907*
DI	0.3485	0.4093	0.6482*
FORM C	0.2336	—	0.5059
FORM S	0.5994*	-0.3340	0.4387
PROP C	0.0351	—	0.7223**
CRIMP C	0.5207	—	0.7128**
HCl C	0.1947	-0.5272	0.6191*
HCl S	0.9307***	-0.6060*	-0.3397
PHOS C	0.2095	-0.1653	0.7139**
PHOS S	0.8712***	-0.4443	0.1213

Table 3. Main effect treatment means (n = 18), and significance levels for the effects of treatment, time and their interaction in two-way ANOVA. The 5% LSD is shown. Means followed by the same letter are not significantly different at the 5% level as analysed by the Tukey multiple comparison test.

treatment		change in fresh weight (g/50g sample)	change in water content (g/50g sample)	change in dry weight (g/50g sample)	final DM content (%)	juice expressed (ml/50g sample)
T0					26.35	
acid 0.05		-7.94 a	-2.21 b	-5.77 ab	19.31 b	4.5 b
acid 0.01		-9.45 a	-3.37 b	-6.10 a	19.83 b	5.8 c
acid 0.02		-9.72 a	-3.62 b	-6.14 a	20.35 b	6.4 c
air		-8.42 a	-8.40 a	0.05 c	32.36 c	0 a
saline		5.95 b	10.82 c	-4.90 b	15.12 a	0 a
P	treatment	<0.001	<0.001	<0.001	<0.001	<0.001
	time	<0.001	<0.001	<0.001	<0.001	<0.001
	treatment×time	<0.001	<0.001	<0.001	<0.001	<0.001
LSD 5%	treatment	1.673	1.724	0.667	1.419	0.66

Figure 1.

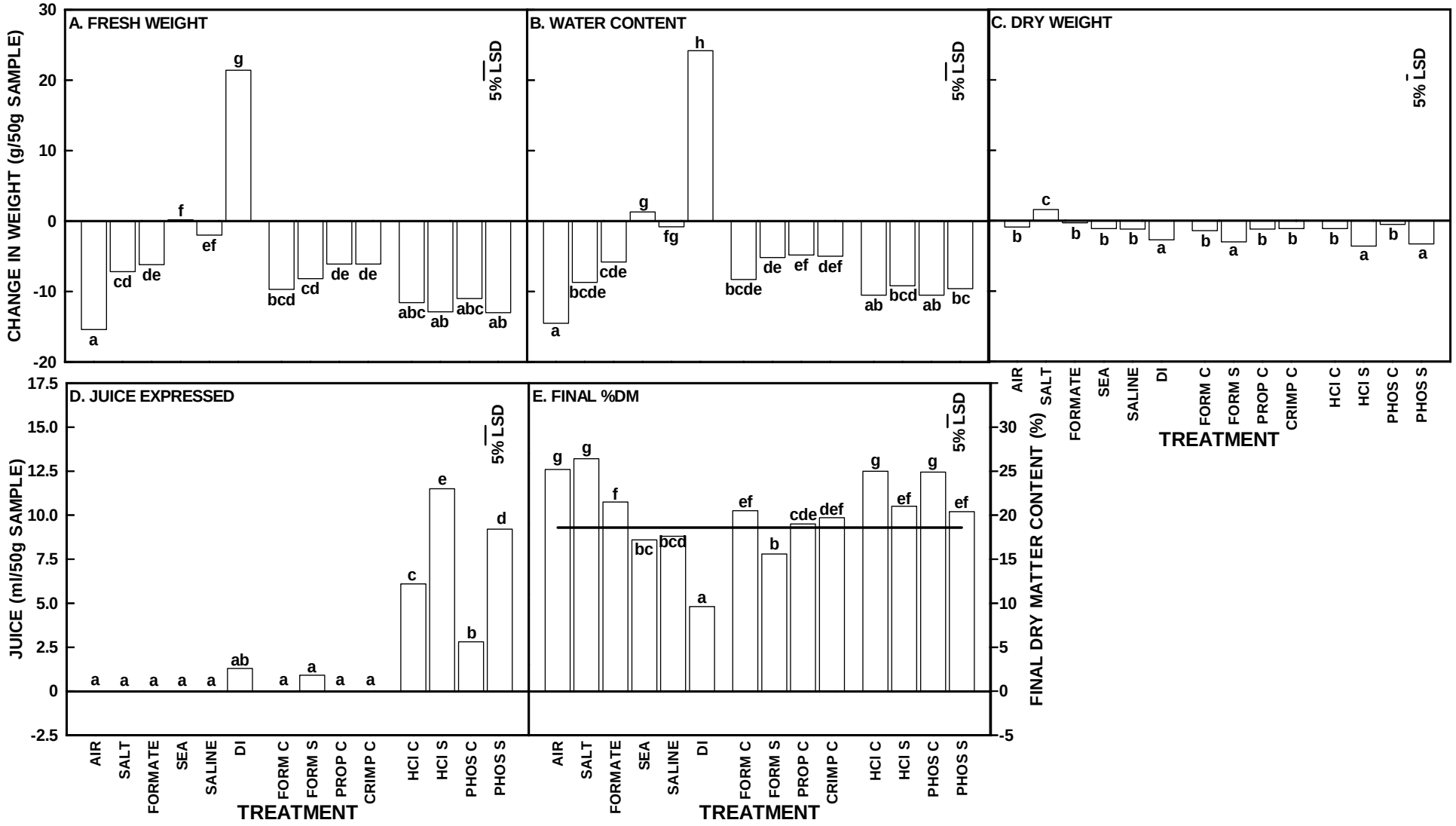

A. FRESH WEIGHT
B. WATER CONTENT
C. DRY WEIGHT
D. JUICE EXPRESSED
E. FINAL %DM
5% LSD
CHANGE IN WEIGHT (g/50g SAMPLE)
JUICE (ml/50g SAMPLE)
FINAL DRY MATTER CONTENT (%)
TREATMENT
AIR
SALT
FORMATE
SEA
SALINE
DI
FORM C
FORM S
PROP C
CRIMP C
HCl C
HCl S
PHOS C
PHOS S

Figure 2.

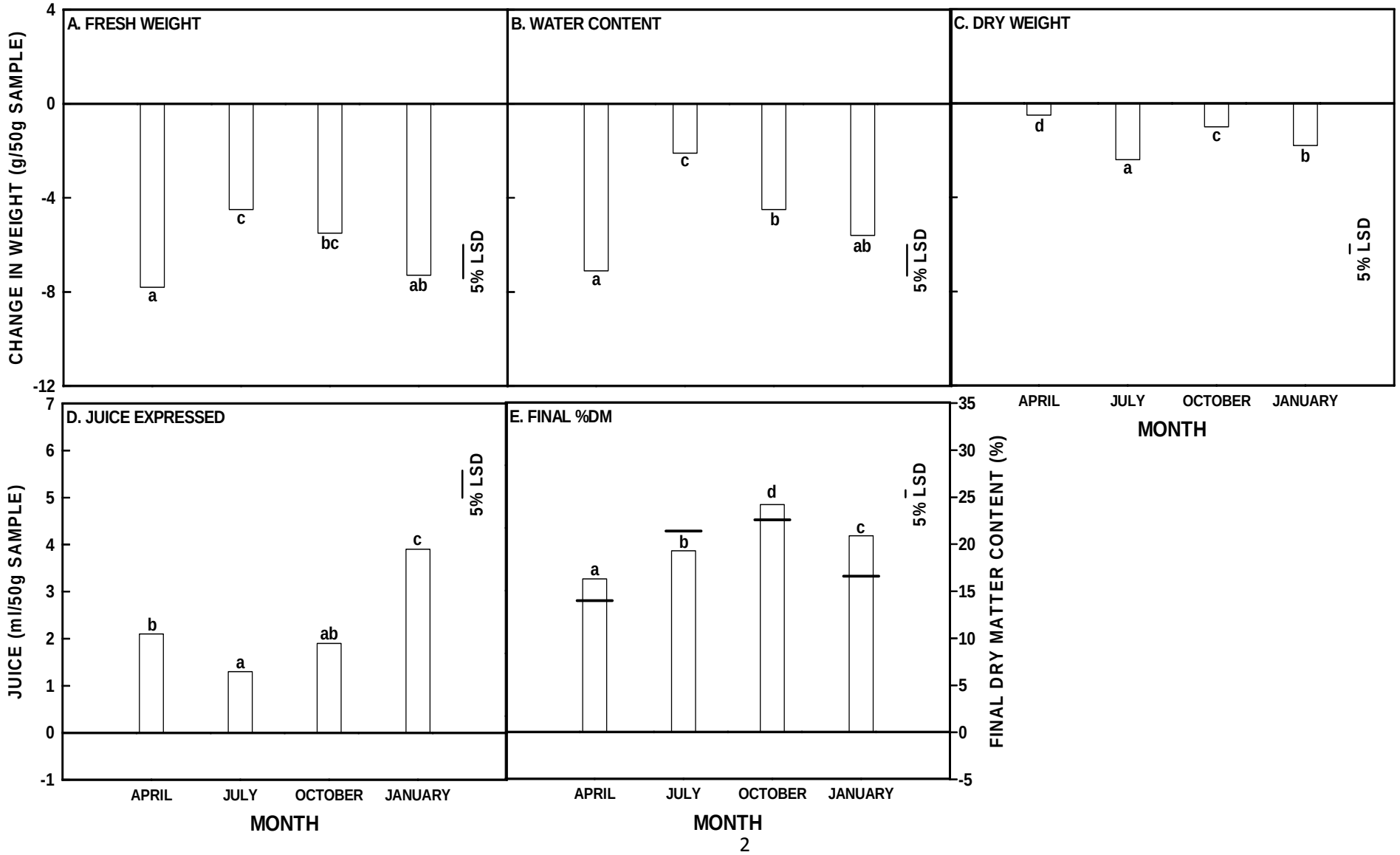

A. FRESH WEIGHT
B. WATER CONTENT
C. DRY WEIGHT
D. JUICE EXPRESSED
E. FINAL %DM
CHANGE IN WEIGHT (g/50g SAMPLE)
JUICE (ml/50g SAMPLE)
FINAL DRY MATTER CONTENT (%)
5% LSD
APRIL
JULY
OCTOBER
JANUARY
MONTH

Figure 3.

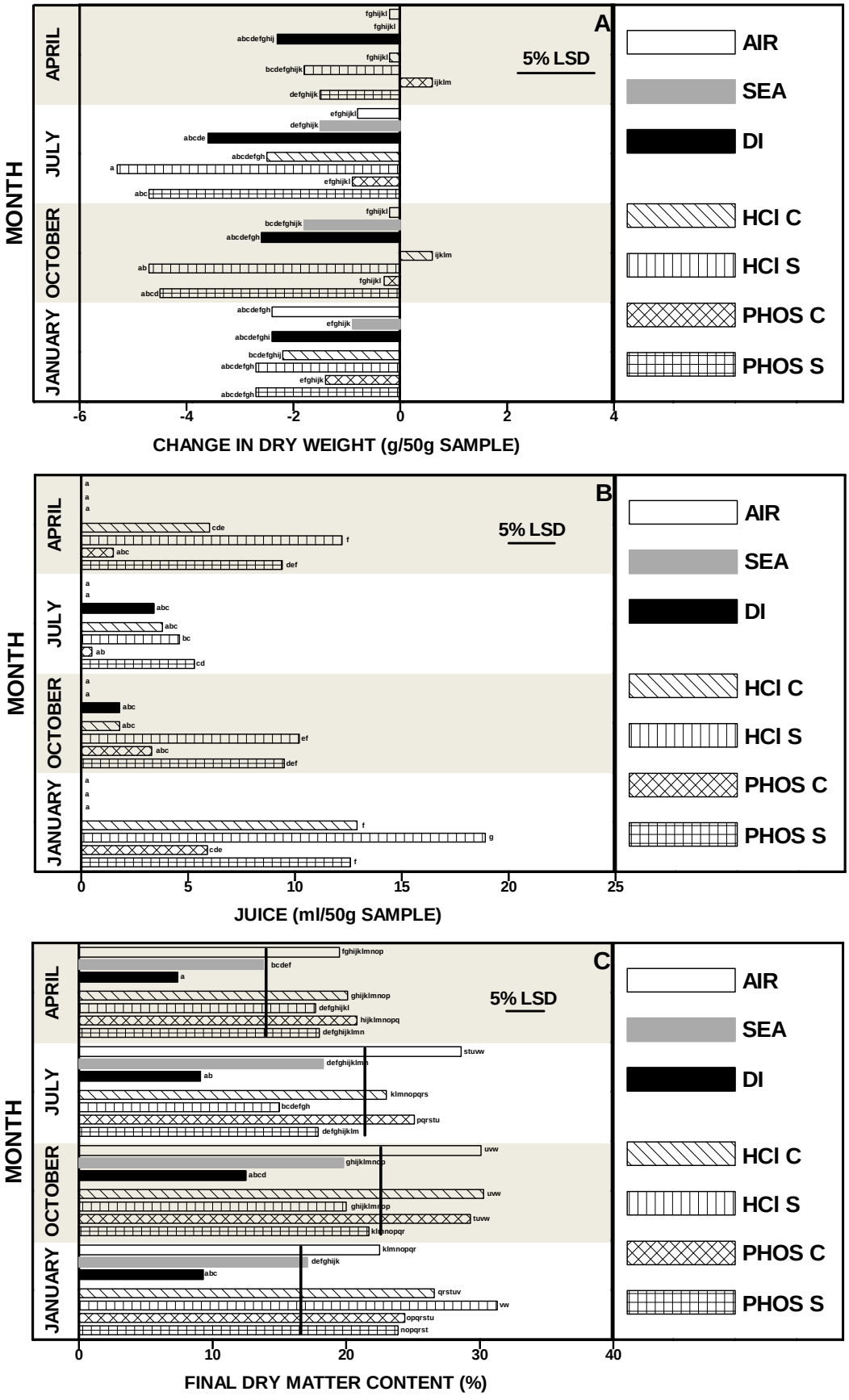

A
5% LSD
MONTH
APRIL
JULY
OCTOBER
JANUARY
AIR
SEA
DI
HCl C
HCl S
PHOS C
PHOS S
-6
-4
-2
0
2
4
CHANGE IN DRY WEIGHT (g/50g SAMPLE)
B
5% LSD
0
5
10
15
20
25
JUICE (ml/50g SAMPLE)
C
5% LSD
0
10
20
30
40
FINAL DRY MATTER CONTENT (%)

Figure 4.

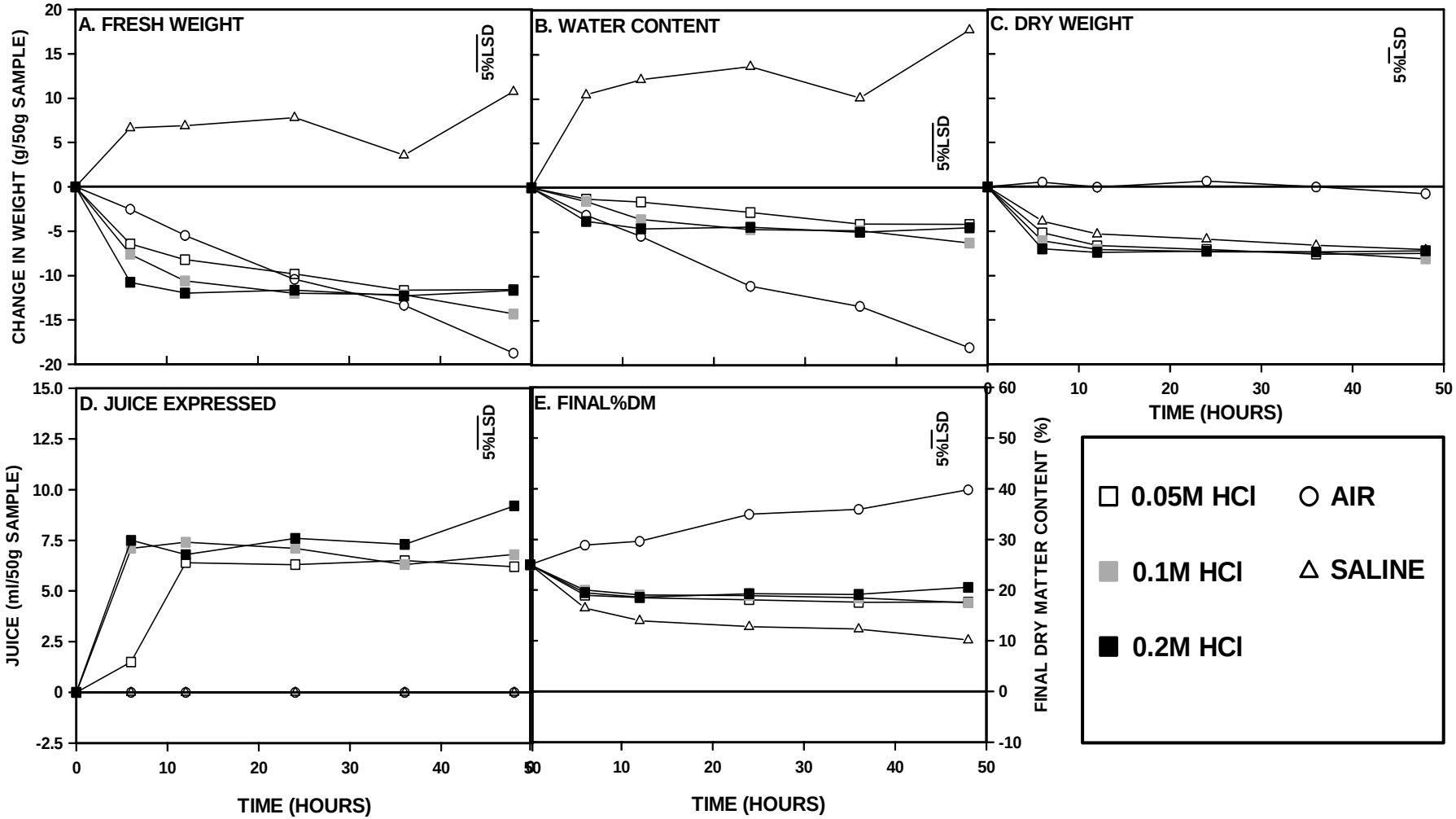
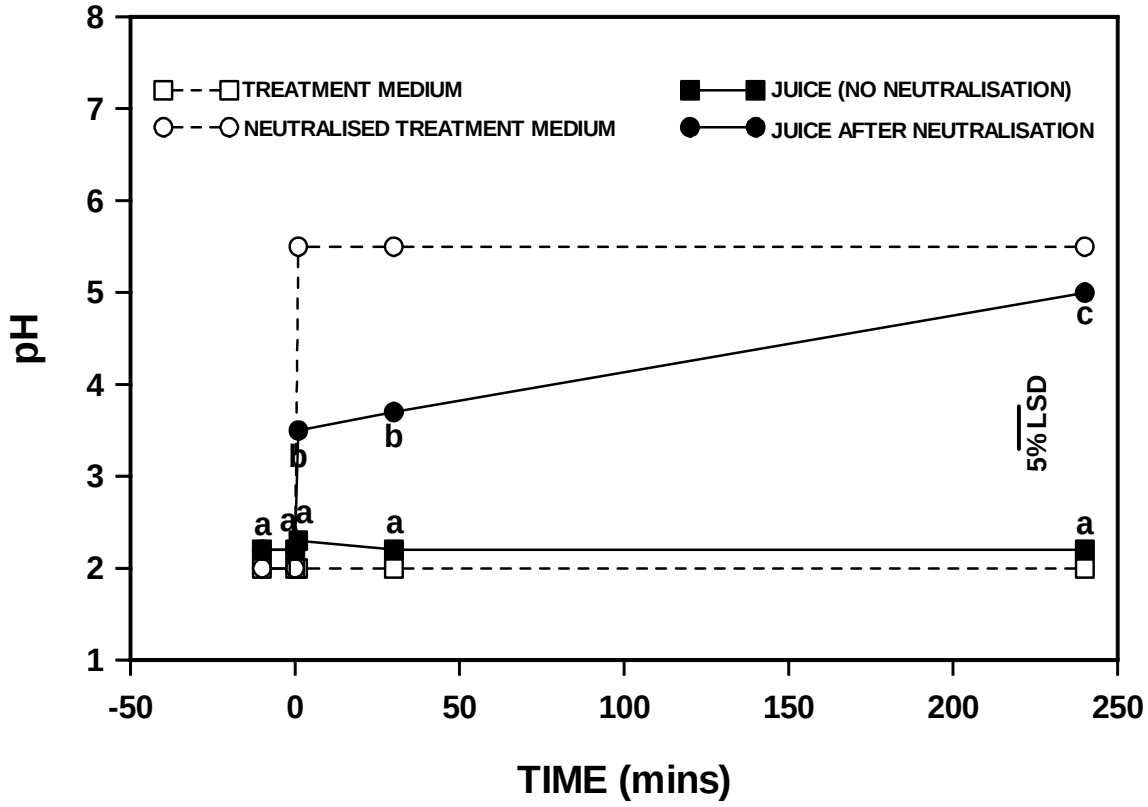

A. FRESH WEIGHT
B. WATER CONTENT
C. DRY WEIGHT
D. JUICE EXPRESSED
E. FINAL%DM
5%LSD
CHANGE IN WEIGHT (g/50g SAMPLE)
JUICE (ml/50g SAMPLE)
FINAL DRY MATTER CONTENT (%)
TIME (HOURS)
0.05M HCl
0.1M HCl
0.2M HCl
AIR
SALINE

Figure 5.


TREATMENT MEDIUM
NEUTRALISED TREATMENT MEDIUM
JUICE (NO NEUTRALISATION)
JUICE AFTER NEUTRALISATION
pH
TIME (mins)
5%LSD
a
b
c